RESEARCH COMMUNICATION

Muscle-specific overexpression of lipoprotein lipase in transgenic mice results in increased α-tocopherol levels in skeletal muscle

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Lipoprotein lipase (LPL) has been implicated in the delivery of chylomicron-located α-tocopherol (α-TocH) to peripheral tissues. To investigate the role of LPL in the cellular uptake of α-TocH in peripheral tissue *in vivo*, three lines of transgenic mice [mouse creatine kinase- (MCK) L, MCK-M and MCK-H] expressing various amounts of human LPL were compared with regard to α-TocH levels in plasma, skeletal muscle, cardiac muscle, adipose tissue and brain. Depending on the copy number of the transgene, LPL activity was increased 3- to 27-fold in skeletal muscle and 1.3- to 3.7-fold in cardiac muscle. The intracellular levels of α-TocH in skeletal muscle were significantly increased in MCK-M and MCK-H animals and correlated highly with the tissue-specific LPL activity ($r = 0.998$). The highest levels were observed in MCK-H (21.4 nmol/g) followed by MCK-M (13.3 nmol/g) and MCK-L (8.2 nmol/g) animals when compared with control mice (7.3 nmol/g). Excellent correlation was also observed between intracellular α-TocH and non-esterified fatty acid (NEFA) levels ($r = 0.998$). Although LPL activities in cardiac muscle were also increased in the transgenic mouse lines, α-TocH concentrations in the heart remained unchanged. Similarly, α-TocH levels in plasma, adipose tissue and brain were unaffected by the tissue specific overexpression of LPL in muscle. The transgenic model presented in this report provides evidence that the uptake of α-TocH in muscle is directly dependent on the level of LPL expression *in vivo*. Increased intracellular α-TocH concentrations with increased triglyceride lipolysis and NEFA uptake might protect the myocyte from oxidative damage during increased β-oxidation.

INTRODUCTION

Vitamin E, a major lipid-soluble, chain-breaking antioxidant is an important factor protecting unsaturated fatty acid residues against (per)oxidative damage [1]. It is an essential nutrient and the major symptoms of vitamin E deficiency are neurological dysfunction, muscular weakness and reproductive failure [2,3]. Vitamin E is a generic term for the naturally occurring tocopherols and tocotrienols and of these, α-tocopherol (α-TocH) has the highest biological activity. Dietary vitamin E is taken up by intestinal cells and resecreted in chylomicrons in proportion with the concentration of the naturally occurring tocopherols in the diet [4,5]. Circulating chylomicrons are catabolized by lipoprotein lipase (LPL; EC 3.1.1.34), with muscle and adipose tissue being the major sites of LPL expression [6]. LPL hydrolyses triacylglycerols (TG) from chylomicrons thereby generating non-esterified fatty acids (NEFA) for subsequent tissue uptake and metabolism. In addition to its function as lipolytic enzyme, several groups have shown that LPL can anchor TG-rich proteins and LDL to the surface of a variety of cells [7–9], increasing surface binding and degradation of these lipoproteins [10–12] by mechanisms involving the LDL-receptor-related protein and the very-low-density lipoprotein (VLDL) receptor [13–15]. Following LPL-mediated hydrolysis of chylomicrons, core remnants are taken up by the liver and part of the lipid constituents are resecreted in newly synthesized VLDL. Studies *in vivo* have demonstrated preferential enrichment of VLDL with RRR-α-tocopherol, a process facilitated by a hepatic α-tocopherol binding/transfer protein (α-TTP) [16–21].

Upon secretion into plasma, VLDL is catabolized by LPL and hepatic triacylglycerol lipase and it has been suggested that these lipolytic enzymes contribute to the distribution of α-TocH between different lipoproteins and tissues. Accordingly, the tocopherol content of adipose tissue from a patient suffering from Type I LPL deficiency was distinctly lower than that of normal subjects [22]. In such cases the mechanisms for tissue uptake of α-TocH are incapacitated. One important step during tissue uptake of α-TocH is apo-lipoprotein B/E-receptor mediated endocytosis of LDL particles [23]. However, Watanabe rabbits lacking functional LDL receptors have normal tissue concentrations of α-TocH [23] and therefore additional mechanisms for α-TocH uptake must exist. It has been suggested that LPL could facilitate α-TocH uptake by the enzyme having a bridging function between lipoproteins and cell membrane glycosaminoglycans. Indeed it has been shown *in vitro* that LPL enhances α-TocH transfer from lipid emulsions to erythrocytes and fibroblasts [24]. Other studies have revealed that LPL facilitates the (selective) uptake of cholesteryl esters and ethers, and retinoids [25,26].

Hence, the present study was designed to investigate the effects of LPL overexpression on the α-TocH status of various tissues with special emphasis on skeletal and cardiac muscle, the sites of specific LPL overexpression.

EXPERIMENTAL

Generation of transgenic mice

The detailed procedure of transgene generation is described elsewhere [27]. Briefly, an LPL minigene was fused with the regulatory sequences of the mouse creatine kinase (MCK) gene.

Abbreviations used: FAME, fatty acid methyl esters; HDL, high-density lipoproteins; LDL, low-density lipoproteins; LPL, lipoprotein lipase; MCK, mouse creatine kinase; NEFA, non-esterified fatty acids; TG, triacylglycerols; α-TocH, α-tocopherol; VLDL, very-low-density lipoproteins.

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The chimaeric DNA construction was injected into the male pronucleus of fertilized eggs from superovulated females and transferred into the oviducts of surrogate females. Three transgenic mouse lines were established: a low (MCK-L), medium (MCK-M) and high (MCK-H) expression line. All animals were kept on a standard laboratory chow.

LPL activity

Epididymal fat pads, heart and thigh muscle samples were surgically removed and transferred to ice-cold tubes containing 1 ml of Dulbecco's modified Eagle's medium/2 % (w/v) BSA/2 units/ml of heparin. The samples were minced with scissors and incubated at 37 °C for 1 h. The activity of LPL in muscle and adipose tissue was assayed as described previously [28].

Tissue sampling and homogenization

After removal, the tissue samples (80–250 mg) were immediately frozen in liquid N_2 to minimize the risk of inadvertent oxidation during handling of the samples [29]. The frozen samples were homogenized in a mortar in liquid N_2 and transferred to preweighed Pyrex tubes with Teflon screw caps. The samples were extracted three times in a biphasic extraction system of water and $CHCl_3$/MeOH [2:1 (v/v) containing 0.2 % (v/v) acetic acid] as described previously [30]. The combined lipid extracts were dried under N_2 and used for NEFA or tocopherol analysis. Microscopy and histochemistry of fresh muscle specimens for fibre-type classification was performed [27].

Fatty acid analysis

To avoid overestimation of NEFA concentrations, lipid extracts were pre-separated by TLC followed by GC analysis of fatty acid methyl esters (FAME) [31]. The band corresponding to the NEFA fraction was scraped off under Ar_2 and transmethylated with BF_3/MeOH in toluene [32]. NEFA recovery from TLC plates was between 96 and 105 % when heptadecanoic and linoleic acid were used as standards. Separation of FAME was performed on a HP20M column (25 m, 0.35 mm internal diameter) using a HP5890 gas chromatograph equipped with a flame ionization detector. The split ratio was approx. 5:1. Concentrations of the individual fatty acids were calculated by peak-area comparison with the internal standard (heptadecanoic acid).

α-TocH analysis

Excised tissues were homogenized and extracted exactly as described above. Lipid extracts were dried under Ar_2, redissolved in the mobile phase used for the HPLC analysis and centrifuged at 30000 ***g***. before HPLC analysis. Separation of α-TocH was performed on a Nucleosil GoldPak 25 cm silica column using hexane/1 % (v/v) ethanol as the mobile phase at a flow rate of 1 ml/min [33]. Detection was by fluorescence spectroscopy with the detector set at 292 nm (excitation) and 335 nm (emission). Concentrations were calculated by peak-area comparison with external standards of known concentration.

Density-gradient ultracentrifugation of plasma

To localize α-TocH in the lipoprotein classes of control and transgenic animals, plasma pools were separated by ultracentrifugation in a two-step density gradient (Beckman TL 120 benchtop centrifuge) as described previously [34]. Tris/HCl (10 mM, pH 7.4; density 1.006 g/ml, 3.7 ml) was underlayered with density-adjusted plasma (1.7 ml, density 1.21 g/ml) and centrifuged at 417000 ***g*** at r_{av} for 2 h at 15 °C. Subsequently, 500 μl fractions were collected from the bottom of the gradient and 200 μl of ethanol and 200 μl of water were added to each fraction. The samples were mixed by vortexing and the neutral lipid fraction was extracted into 200 μl of hexane. Phase separation was achieved by centrifugation at 30000 ***g*** and a 20 μl portion of each upper phase was analysed by HPLC. The α-TocH content was quantified as described above.

RESULTS

LPL activities and NEFA concentrations in skeletal muscle

Three independent transgenic mouse lines were created as reported previously [27]. LPL activities in skeletal muscle of controls, MCK-L, MCK-M and MCK-H animals are shown in Table 1. LPL activities in skeletal muscle were increased 3.3-, 11.7- and 27-fold in MCK-L, MCK-M and MCK-H animals respectively when compared with control mice. As a result of LPL overexpression in skeletal muscle we observed an increase in NEFA concentrations (nmol/g of wet tissue, means ± S.D.) from 566 ± 203 in control animals to 718 ± 273 in MCK-L, 1432 ± 325 in MCK-M and 2407 ± 685 in MCK-H animals (Table 1). NEFA levels measured in the control animals are in agreement with values reported for different muscle types in rats [35,36].

Effect of muscle-specific overexpression of LPL on plasma α-TocH levels

The plasma α-TocH concentrations (means ± S.D.) were 5.8 ± 0.79, 4.9 ± 1.59, 4.5 ± 0.58 and 4.3 ± 0.95 μM in control, MCK-L, MCK-M and MCK-H animals respectively ($n = 10$ for controls and $n = 8$ for transgenic animals), slightly lower values than reported previously for rat plasma [37,38]. These analyses revealed a tendency towards a slight decrease in circulating α-TocH concentrations with increasing number of transgene copies but the effect was not statistically significant.

Inter-lipoprotein distribution of α-TocH

To investigate if overexpression of LPL in transgenic animals caused changes in the inter-lipoprotein distribution of α-TocH, pooled plasma samples of controls, MCK-L, MCK-M and MCK-H animals ($n = 3$) were subjected to density-gradient

Table 1 Effects of LPL transgene copy number on heparin-releasable enzyme activities and NEFA levels in skeletal muscle

Fibre-type classification revealed the presence of predominantly (⩾ 99%) white (fast twitch, type II) muscle elements. Values represent the means ± S.D for five animals for LPL activity and eight animals for NEFA estimation.

	Copy number	LPL activity (μM NEFA/h per g of tissue)	NEFA (nmol/g tissue)
Controls	–	1.1 ± 0.42	566 ± 202.8
MCK-L	3	3.6 ± 0.45[a]	718 ± 272.9
MCK-M	10	12.9 ± 3.20[a]	1432 ± 324.8[a]
MCK-H	20	29.8 ± 8.89[a]	2407 ± 685.2[a]

[a] $P < 0.01$

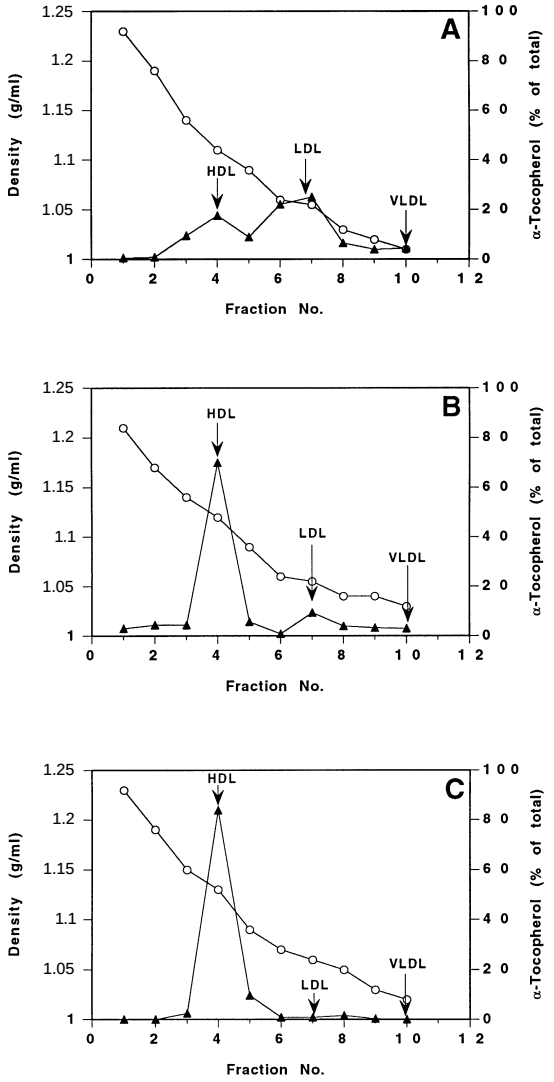

Figure 1 Inter-lipoprotein distribution of α-TocH in plasma

(**A**) Pooled human plasma, (**B**) pooled plasma of control mice and (**C**) pooled plasma of MCK-H animals. Lipoproteins were isolated by ultracentrifugation in a two-step density gradient; the gradient (5.1 ml total volume) fractions were collected from the bottom of the tube in 500 μl portions and transferred to preweighed Eppendorf tubes for density estimation. Subsequently these samples were extracted and analysed for α-TocH concentration. 100% corresponds to 22.5 μM (**A**), 6.1 μM (**B**) and 5.2 μM (**C**) respectively. ○, Density; ▲, α-tocopherol.

ultracentrifugation. The fractions were collected from the bottom of the tube in ten fractions which were analysed for α-TocH. Representative results of these experiments are shown in Figure 1. The density distribution of α-TocH in normal mouse plasma is quite different from that observed in humans where α-TocH is distributed in an almost 1:1 ratio between high- (HDL) and low-density lipoproteins (LDL) (Figure 1A, plasma pool obtained from 6 fasting human donors, α-Toc, 22.5 μM). In normal mice, 80–95% of total plasma α-TocH was recovered in the density region 1.10–1.15 g/ml, corresponding to mouse HDL. Muscle-specific overexpression of LPL resulted in a slight shift of α-TocH from the LDL to the HDL fraction. In the LDL fraction of controls 13±6% of α-TocH was recovered, whereas in MCK-H animals 6±1.6% of total α-TocH was located in the LDL density fraction. Results of a representative experiment are shown in Figures 1(B) and 1(C).

Table 2 Effect of tissue-specific LPL overexpression on α-TocH levels in skeletal and cardiac muscle, adipose tissue and brain

Control, MCK-L, MCK-M and MCK-H animals were maintained on standard laboratory chow. Values represent the means±S.D. in nmol/g wet tissue.

Tissue	Controls (n = 13)	MCK-L (n = 9)	MCK-M (n = 9)	MCK-H (n = 7)
Skeletal muscle	7.3±2.74	8.3±2.07	13.3±4.70[a]	21.4±8.11[a]
Cardiac muscle	22.8±4.61	20.7±3.52	22.8±2.71	20.1±2.4
Adipose tissue	64.4±35.3	33.6±13.6	47.4±30.3	28±14.8
Brain[b]	17.6±3.68	15.1±2.57	17.3±2.01	
	10.4±1.11			9.36±0.78

[a] $p < 0.01$

[b] Controls and transgenic animals were age-matched; the MCK-H animals used for these analyses were 2 months and the MCK-L and MCK-M animals were 4 months old.

α-TocH levels in skeletal muscle, cardiac muscle, adipose tissue and brain of control and transgenic animals

Table 2 summarizes the α-TocH concentrations found in skeletal muscle (fast twitch, type II fibres), cardiac muscle, adipose tissue and brain of age-matched control, MCK-L, MCK-M and MCK-H animals. Compared with controls, steady-state concentrations of α-TocH in thigh muscle were increased significantly in MCK-M (1.8-fold) and MCK-H (2.9-fold) animals (Table 2, $P < 0.01$, compared with controls). The α-TocH content of muscle observed in our control animals is similar to that found for rat muscle specimens (7.3 compared with 5.1 nmol/g wet tissue [37]). From the data shown in Table 2 it is evident that muscle-specific overexpression of LPL in mice resulted in a pronounced increase in skeletal muscle α-TocH content which was dependent on the degree of LPL overexpression. When the α-TocH levels were compared with skeletal muscle-specific LPL activity, a linear relationship between the increase in heparin-releasable LPL activity and α-TocH concentration in the corresponding muscle specimens was observed (Figure 2A, $r = 0.998$). In agreement with the enhanced lipolytic activity, the increase in skeletal muscle NEFA concentration was accompanied by a linear increase in α-TocH levels (Figure 2B, $r = 0.998$).

As reported previously, the muscle creatine kinase promoter results in a highly tissue-specific overexpression of LPL, primarily in skeletal muscle [27]. However, a moderate increase (between 1.3- and 3.7-fold) was observed also in cardiac muscle [27]. This increase in cardiac muscle-specific LPL activity did not result in increased α-TocH levels in the heart. The α-TocH concentrations (means±S.D.) in cardiac muscle specimens obtained from controls and transgenic animals were almost identical (22.8±4.6, 20.7±3.5, 22.8±2.7 and 20.1±2.4 nmol/g wet tissue for controls, MCK-L, MCK-M and MCK-H respectively; Table 2).

The α-TocH levels in adipose tissue in the three transgenic lines did not vary significantly from control levels (Table 2) and were the highest of all tissue specimens analysed (64.4±35.3, 33.6±13.6, 47.4±30.3 and 28.0±14.8 nmol/g wet tissue for controls, MCK-L, MCK-M and MCK-H animals respectively), which supports the proposed function of adipose tissue as one of the major α-TocH storage depots [39]. Brain α-TocH concentrations were not significantly different between control and

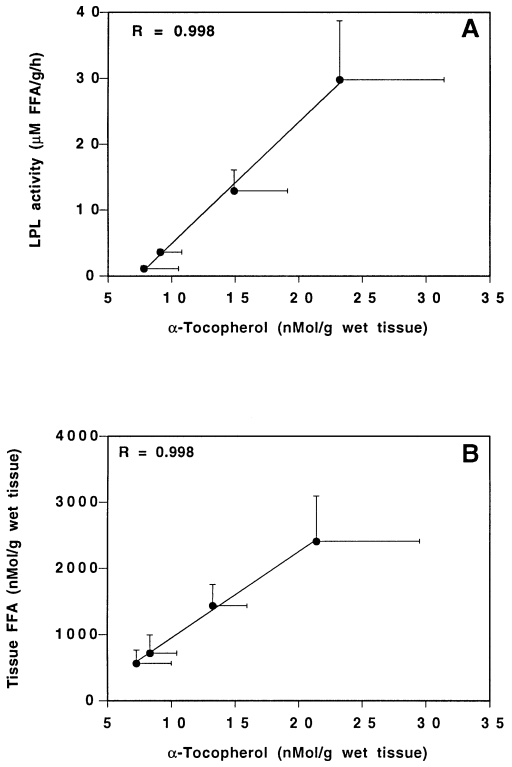

Figure 2 Comparison of heparin-releasable LPL activity, NEFA (FFA) and α-TocH concentrations in skeletal muscle of control and transgenic animals

(**A**) Comparison of heparin-releasable LPL activity and α-TocH concentration in skeletal muscle of control and transgenic animals. Thigh muscles (type II fibers) were removed surgically and LPL activity and α-TocH concentrations were estimated. Tissue homogenization prior to lipid extraction and HPLC analysis was performed in liquid N_2. Values represent means ± S.D. ($n = 5$). (**B**) Comparison of NEFA and α-TocH concentrations in skeletal muscle of control and transgenic animals. Thigh muscles (type II fibers) were removed surgically and immediately homogenized in liquid N_2. Lipid extracts were separated into lipid subclasses by TLC and the band corresponding to the NEFA fraction was removed. The NEFA were methylated directly on the TLC adsorbent using BF_3/MeOH. The corresponding FAME were separated by GLC as described in the Experimental section. Values represent the means ± S.D. ($n = 8$).

transgenic animals (Table 2). Brain specimens obtained from 8-week old mice contained, on average, 9.9 nmol α-TocH/g wet tissue and the corresponding value for older animals (16 weeks) was 16.7 nmol/g wet tissue.

DISCUSSION

The results of cell culture experiments implicate LPL in the delivery of α-TocH from triglyceride-rich lipoproteins to peripheral tissues. For example, Traber and Kayden [22] have shown that the addition of bovine LPL enhanced the net transfer of fatty acids and tocopherols to erythrocytes and fibroblasts. Additional evidence that LPL is involved in α-TocH uptake *in vivo* was obtained from patients suffering from familial chylomicronaemia caused by the inherited absence of the enzyme, these individuals had decreased or low normal levels of α-TocH in adipose tissue [22,40]. However, to date no information is available on the role of LPL in the uptake of α-TocH in muscle, although it is a major site of LPL expression in the body.

To test the hypothesis that increased LPL expression in muscle can induce α-TocH accumulation, transgenic mouse lines expressing various amounts of human LPL in skeletal and cardiac muscle were studied. In skeletal muscle α-TocH levels rose significantly with increasing LPL activity. When α-TocH concentrations were compared with the tissue LPL activity or the intracellular NEFA concentrations, a high correlation was observed in these parameters. These results provided strong evidence *in vivo* that LPL can enhance the uptake of α-TocH in skeletal muscle and are in accordance with earlier studies in other cell types *in vitro* demonstrating LPL-dependent uptake of α- and γ-TocH [22].

The fact that α-TocH is also provided to cells and tissues that do not express LPL or lack the enzyme due to a genetic defect, suggests that other mechanisms exist, in addition to LPL, for the cellular uptake of α-TocH. Since the majority of α-TocH is lipoprotein-associated, these alternative mechanisms include the receptor-mediated endocytosis of lipoproteins [22], and the selective cellular uptake of α-TocH, which is independent of receptor-mediated, whole-particle uptake [23,41]. However, the presence of LPL apparently represents a major determinant of the relative amount found in a given tissue. Accordingly it was shown that rat tissues expressing LPL, such as adipose tissue and muscle, contain almost 65% of the total amount of α-TocH measured in ten different organs [37].

The molecular mechanisms by which overexpression of LPL facilitates α-TocH uptake remain to be elucidated. Overexpression of LPL leads to enhanced chylomicron and VLDL lipolysis followed by the uptake of lipolysis products such as NEFA and α-TocH. The high correlation between LPL activity and the intracellular NEFA and α-TocH levels in skeletal muscle suggest a similar uptake mechanism for both. Several mechanisms have been proposed for cellular NEFA uptake, including transport-protein mediated processes or direct, protein-independent uptake [42–44]. Similar mechanisms might exist to facilitate α-TocH uptake and need to be addressed. Whether LPL mediates α-TocH uptake by the proposed bridging function of the enzyme or by lipoprotein particle-independent, selective uptake of α-TocH remains to be elucidated. Whereas overexpression of LPL resulted in increased α-TocH levels in skeletal muscle, cardiac α-TocH levels remained unaffected. This could be explained by the rather moderate overexpression of the LPL minigene in cardiac muscle. In fact, the highest activity was a 3.7-fold overexpression (MCK-H animals) which might not be sufficient to result in a significant increase in steady-state intracellular α-TocH levels. Because of the highly tissue-specific properties of the MCK promoter, LPL activities were not altered in any tissue other than muscle. Accordingly, α-TocH concentrations in tissues such as adipose or brain were identical with those in the corresponding tissues of control animals. In the brain, however, significantly higher α-TocH levels were observed in the older animals and could suggest an age-dependent increase as described in an earlier report [38].

Overexpression of human LPL in skeletal and cardiac muscle of transgenic animals causes premature death from severe myopathy [27]. It is generally assumed that α-TocH deficiency can result in the development of myopathies, probably because of increased lipid peroxidation in the affected tissues [45]. Therefore our findings might reflect an extreme physiological situation of increased β-oxidation and oxidative phosphorylation in which increased cellular α-TocH concentrations serve as a defense mechanism to prevent excessive oxidative damage. As shown previously, increased NEFA uptake because of LPL overexpression in skeletal muscle causes a marked increase in the number of mitochondria and peroxisomes [27]. It is well established that these cellular organelles are implicated in the production of reactive oxygen species. In mitochondria, O_2^- anions are produced during oxidative phosphorylation, with complexes I and III being the major sites of free-radical pro-

duction [46,47]. In peroxisomes, β-oxidation results in the formation of H_2O_2 [48]. In a situation of massive NEFA excess both processes potentially contribute to the formation of lipid-peroxidation products and the amount of α-TocH or increased catalase activity is insufficient to suppress the initiation of lipid peroxidation. This might lead ultimately to the development of the myopathy in these animals. Since LPL represents the major determinant of the amount of NEFA that enters skeletal myocytes it makes physiological sense that, simultaneously with other factors, it controls the uptake of one of the major radical scavengers, thus maintaining the integrity of the antioxidative defence system in muscle.

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REFERENCES

1 Niki, E. (1987) Chem. Phys. Lipids **44**, 227–253
2 Muller, D. P. R. (1995) Redox Report **1**, 239–245
3 Guggenheim, M. A., Ringel, S. P. and Silverman, A. (1982) Ann. N.Y. Acad. Sci. **393**, 84–95
4 Traber, M. G., Burton, G. W., Ingold, K. U. and Kayden, H. J. (1990) J. Lipid Res. **31**, 675–685
5 Gallo-Torres, H. (1970) Lipids **5**, 379–384
6 Nilsson-Ehle, P., Garfinkel, A. S. and Schotz, M. (1980) Annu. Rev. Biochem. **49**, 667–693
7 Skottova, N., Savonen, R., Lookene, A., Hultin, M. and Olivecrona, G. (1995) J. Lipid Res. **36**, 1334–1344
8 Krapp, A., Zhang, H., Ginzinger, D., Liu, M.-S., Lindberg, A., Olivecrona, G., Hayden, M. R. and Beisiegel, U. (1995) J. Lipid Res. **36**, 2362–2373
9 Mann, W. A., Meyer, N., Weber, W., Rinninger, F., Greten, H. and Beisiegel, U. (1995) Eur. J. Clin. Invest. **25**, 880–882
10 Williams, K. J., Fless, G. M., Petrie, K. A., Snyder, M. L., Brocia, R. W. and Swenson, T. L. (1992). J. Biol. Chem. **267**, 13284–13292
11 Eisenberg, S., Sehayek, E., Olivecrona, T. and Vlodavsky, I. (1992) J. Clin. Invest. **90**, 2013–2021
12 Rumsey, S. C., Obunike, J. C., Arad, Y., Deckelbaum, R. J. and Goldberg, I. J. (1992) J. Clin. Invest. **90**, 1504–1512
13 Beisiegel, U., Weber, W. and Bengtsson-Olivecrona, G. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 8342–8346
14 Williams, S. E., Inoue, I., Tran, H., Fry, G. L., Pladet, M. W., Iverius, P.-H., Lalouel, J.-M., Chappell, D. A. and Strickland, D. K. (1994) J. Biol. Chem. **269**, 8653–8658
15 Takahashi, S., Suzuki, J., Kohno, M., Oida, K., Tamai, T., Miyabo, S., Yamamoto, T. and Nakai, T. (1995) J. Biol. Chem. **270**, 15747–15754
16 Traber, M. G. (1994) Free Radicals Biol. Med. **16**, 229–239
17 Catignani, G. L. and Bieri, J. G. (1977) Biochim. Biophys. Acta **497**, 349–357
18 Dutta-Roy, A. K., Leishman, D. J., Gordon, M. J., Campbell, F. M. and Duthie, G. D. (1993) Biochem. Biophys. Res. Commun. **196**, 1108–1112
19 Arita, M., Sato, Y., Miyata, A., Tanabe, T., Takahashi, E., Kayden, H. J., Arai, H. and Inoue, K. (1995) Biochem. J. **306**, 437–443
20 Traber, M. G., Sokol, R. J., Kohlschütter, A., Yakota, T., Muller, D. P. R., Dufour, R. and Kayden, H. J. (1993) J. Lipid Res. **34**, 201–210
21 Ouahchi, K., Arita, M., Kayden, H., Hentati, F., Ben Hamida, M., Sokol, R., Arai, H., Inoue, K., Mandel, J-L. and Koenig, M. (1995) Nature Gen. **9**, 141–145
22 Traber, M. G. and Kayden, H. J. (1984) Am. J. Clin. Nutr. **40**, 747–751
23 Cohn, W., Goss-Sampson, M. A., Grun, H. and Muller, D. P. R. (1992) Biochem. J. **287**, 247–254
24 Traber, M. G., Olivecrona, T. and Kayden, H. J. (1985) J. Clin. Invest. **75**, 1729–1734
25 Stein, O., Halperin, G., Leitersdorf, E., Olivecrona, T. and Stein, Y. (1984) Biochim. Biophys. Acta **795**, 47–59
26 Blaner, W. S., Obunike, J. C., Kurlandsky, S. B., Al-Haideri, M., Piantedosi, R., Deckelbaum, R. J. and Goldberg, I. J. (1994) J. Biol. Chem. **269**, 16559–16565
27 Levak-Frank, S., Radner, H., Walsh, A., Stollberger, R., Knipping, G., Hoefler, G., Sattler, W., Weinstock, P. H., Breslow, J. L. and Zechner, R. (1995) J. Clin. Invest. **96**, 976–986
28 Zechner, R. (1990) Biochim. Biophys. Acta **1044**, 20–25
29 Christie, W. W. (1993) in Advances in Lipid Methodology, II (Christie, W. W., ed.) pp. 195–213, The Oily Press, Dundee
30 Bligh, E. G. and Dyer, W. J. (1959) Can. J. Biochem. Physiol. **37**, 911–917
31 Hallaq, J., Becker, T. C., Manno, C. S. and Laposata, M. (1993) Lipids **28**, 355–360
32 Sattler, W., Puhl, H., Hayn, M., Kostner, G. M. and Esterbauer, H. (1991) Anal. Biochem. **198**, 184–190
33 Rhys Williams, A. T. (1985) J. Chromatogr. **341**, 198–201
34 Sattler, W., Mohr, D. and Stocker, R. (1994) Methods Enzymol. **233**, 469–489
35 Gorski, J. (1991) Can. J. Physiol. Pharmacol. **70**, 123–131
36 Hopp, J. F. and Palmer, W. K. (1990) J. Appl. Physiol. **68**, 348–354
37 Bjornboe, A., Bjornboe, G-E.Aa., Bodd, E., Hagen, B. F., Kveseth, N. and Drevon, Ch. A. (1986) Biochim. Biophys. Acta **889**, 310–315
38 Clement, M., Dinh, L., Bourre, J.-M. (1995) Biochim. Biophys. Acta **1256**, 175–180
39 Dutta-Roy, A. K., Gordon, M. J., Campbell, F. M., Duthie, G. G. and James, W. P. T. (1994) J. Nutr. Biochem. **5**, 562–570
40 Traber, H. J. (1983) in Biology of Vitamin E (Porter, R. and Whelan, J., eds.) pp. 70–91, Pittman Books, London.
41 Cohn, W., Gross, P., Grun, H., Loechleitner, F., Muller, D. P. R. and Goss-Sampson, M. A. (1993) in Vitamin E – Its Usefulness in Health and Curing Diseases (Mino, M., Nakamura, H., Diplock, A. T. and Kayden, H. J., eds.) pp. 95–102, S. Karger, Basel
42 Schaffer, J. E. and Lodish, H. F. (1995) Trends Cardiovasc. Med. 5, 218–224
43 Stremmel, W. G. (1988) J. Clin. Invest. **81**, 844–852
44 Wetzel, M. G. and Scow, R. O. (1984) Am. J. Physiol. **246**, C467–C485
45 Thomas, P. K., Cooper, J. M., King, R. H. M., Workman, J. M., Schapira, A. H. V., Goss-Sampson, M. A. and Muller, D. P. R. (1993) J. Anat. **183**, 451–461
46 De Bono, D. P. (1994) Q. J. Med. **87**, 445–453
47 Turrens, J. F. and Boveris, A. (1980) Biochem. J. **191**, 421–427
48 del Rio, A. L., Sandalio, L. M., Palma, J. M., Bueno, P. and Corpas, F. J. (1992) Free Radicals Biol. Med. **13**, 557–580

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