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Protective Effects of α-Tocopherol against Glucose Intolerance and Mitochondrial Damage in Reperfused Rat Liver

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Summary We examined the protective effects of α-tocopherol against glucose intolerance and mitochondrial damage in rat liver after partial *in vivo* ischemia and reperfusion, while glucose was intravascularly supplemented. Rats were given α-tocopherol or vehicle for three consecutive days before the experiment. Left and median lobes of the liver received 90-min ischemia and 60-min reperfusion. Besides plasma levels of glucose, insulin, and lipid peroxide, we also measured oxidative phosphorylation, cytochrome *c* oxidase (CCO) activity, and lipid peroxide levels in mitochondria in both ischemic and non-ischemic lobes of the liver in control and α-tocopherol groups before and at the end of ischemia and after reperfusion. Both plasma glucose and insulin levels after reperfusion were lower in the α-tocopherol-treated group than in the control group. Ischemia markedly suppressed both mitochondrial respiration supported by succinate and CCO activity in the ischemic lobes in both groups. Reperfusion recovered mitochondrial function and CCO activity better in the α-tocopherol-treated group than in the control group, whereas the lipid peroxide levels in both plasma and mitochondria were significantly lower in the α-tocopherol-treated group. In non-ischemic lobes, ischemia-reperfusion slightly accelerated both the oxidative phosphorylation rate (OPR) and CCO activity, whereas lipid peroxide levels were unaffected. The results suggest that glucose intolerance during the early phase of reperfusion was not due to lowered plasma insulin level, but might be related to liver damage, as assessed in terms of mitochondrial functions, in the ischemic lobes. α-Tocopherol could protect against

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mitochondrial damage and glucose intolerance. Oxidative phosphorylation in non-ischemic lobes likely compensated for the decreased mitochondrial activity in the ischemic lobes during ischemia and reperfusion.

Key Words: liver, α-tocopherol, glucose intolerance, mitochondria, ischemia-reperfusion injury

Administration of a large dose of glucose is apt to induce hyperglycemia when liver function is poor [1]. This phenomenon is attributed to lowered glucose metabolism in the liver and to disturbed regulation of plasma glucose levels throughout the body. Impaired liver function is likely due to suppressed mitochondrial activity and reduced cellular ability to use glucose as an energy source [1, 2]. Recent increases in liver surgeries with extensive invasion such as resection and the transplantation have, therefore, demanded improved post-operative management of patients. Improved management may include treatment of patients based on the arterial ketone body ratio (AKBR: [acetoacetate]/[β-hydroxybutyrate]), which is relevant to [NAD^+]/[NADH] in mitochondria, as a nutritional index [3–7]. The initial stage of acute liver injury is associated with a lowered AKBR, which indicates suppressed utilization of glucose in the liver as the principal substrate for ATP synthesis. Furthermore, when secretion of insulin is induced by administration of a large dose of glucose, peripheral adipose tissues may fail to release fatty acids, which are used as the substrate instead of glucose when the liver functions poorly. Therefore, clinically, initial transfusions with low glucose solutions are given and the glucose concentration is gradually raised with special reference to the AKBR [1, 8].

The liver is inevitably exposed to ischemia and subsequent reperfusion during most liver surgeries. It is thought that mitochondrial dysfunction is one of the causes of ischemia-reperfusion injury in the liver [9–15]. Both the electron-transferring and ATP-synthesizing systems in the mitochondrial inner membrane are thought to be vulnerable to hypoxic ischemia and the following reoxygenating reperfusion [16, 17]. On the other hand, cytochrome *c* oxidase (CCO), which is the terminal electron-transfer element (complex IV) in the respiratory chain, is reported to be tolerant of various stresses [17–19], although no report has appeared concerning CCO activity in the liver during *in vivo* ischemia-reperfusion. A variety of radical scavengers has been successfully used to protect the liver from ischemia-reperfusion injury by suppressing the formation of lipid peroxides [10, 20]. Therefore, modification of membrane phospholipids by free radicals during ischemia-reperfusion is thought to contribute to mitochondrial dysfunction [17, 20]. Reperfusion has been reported to accelerate mitochondrial oxidative phosphorylation in the non-ischemic lobes of partial ischemia-reperfusion models, whereas the ischemic lobes show suppressed mitochondrial functions. This fact may indicate that the liver as a whole is capable of maintaining its function even

under hypoxic stress [21]. However, no detailed study has been conducted to examine glucose tolerance and mitochondrial function during ischemia-reperfusion in the presence and absence of radical scavengers during supplementation with glucose.

We examined the effects of α-tocopherol on glucose tolerance and mitochondrial functions in both ischemic and non-ischemic liver lobes of rats in a partial *in vivo* ischemia-reperfusion model in the presence of a large dose of supplemented glucose. The results suggest that α-tocopherol lessened accumulation of lipid peroxides, protected mitochondrial oxidative phosphorylation from ischemia-reperfusion injury, and suppressed the elevation of plasma glucose.

MATERIALS AND METHODS

We followed our college's regulations for animal research, which were based on the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 85-23, 1985).

Experimental protocol. Male Wistar rats (10 weeks old, 330–370 g, Kyushu Animal Supply Co., Kumamoto, Japan) were randomly assigned to a sham group and two experimental groups, i.e., a control group and an α-tocopherol group. All procedures were carried out at 22–25°C and a relative humidity higher than 70%. α-Tocopherol (50 mg/ml *dl*-α-tocopherol) was suspended in a vehicle, composed of 100 mg/ml polyoxyethylene hydrogenated castor oil derivatives-60 mol ether (HCO-60), 100 mg/ml propylene glycol, and 1 mg/ml sodium citrate (pH 3.5). α-Tocopherol (15 mg/kg of body weight/day) was administered by intraperitoneal injection for three consecutive days before the start of the experiment (Fig. 1). The control group received only the vehicle. The animals were fasted for 6 h before the start of experiments and water was given *ad libitum*. Animals were anesthetized by an intraperitoneal injection of sodium pentobarbitone (50 mg/kg body weight). A cannula was set in the femoral artery in the left leg to infuse 0.1 ml of physiologic saline with glucose (35%, w/v) and heparin (200 IU/kg of body weight) in a retrograde direction. The liver hilus was exposed by laparotomy at a median portion. At 20 min after the first glucose infusion, hepatic artery and portal vein to the left and median liver lobes were occluded with vascular clamps, and 0.1 ml of physiologic saline with 35% (w/v) glucose was immediately infused through the cannula. This procedure could render partial ischemia to about 70% of the liver by weight [10, 22]. The remaining parts of the liver could be devoid of ischemia. After 90 min of occlusion, the clamp was removed and blood was reperfused for 60 min. During reperfusion, 0.1 ml of physiologic saline with 35% (w/v) glucose was infused at 1, 15, and 30 min after the removal of the clamp. The abdominal wall was closed during ischemia and reperfusion to reduce dehydration and hypothermia. The sham group, to which α-tocopherol or the vehicle had been administered, received the same cannulation at the femoral artery and abdominal

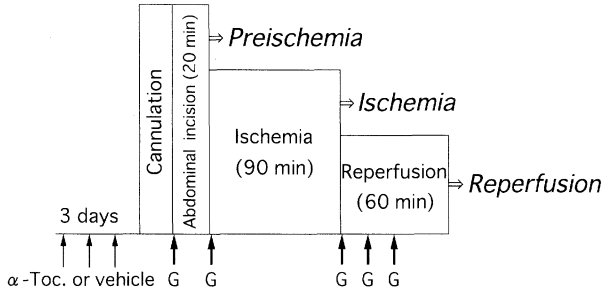

Fig. 1. Experimental protocol. α-Tocopherol (15 mg/kg/day) or the vehicle was administered intraperitoneally for three consecutive days before the experiment was started. One tenth milliliter of physiological saline with 35% (w/v) glucose and heparin (200 IU/kg) were administered through a cannula set at the left femoral artery (arrow marked G). At 20 min after the first infusion of glucose, hepatic artery and portal vein to the left and median liver lobes were occluded (about 70% ischemia), and 0.1 ml of 35% (w/v) glucose solution was immediately infused through the cannula. After 90 min of ischemia, blood was reperfused for 60 min. During the reperfusion, 0.1 ml of glucose solution was infused at 1, 15, and 30 min after removal of the clamp. Rats were sacrificed before ischemia (Preischemia), at the end of ischemia (Ischemia), and after reperfusion (Reperfusion). The sham group received a cannulation and an abdominal incision, but nothing was infused. Mitochondria were isolated before and at the end of ischemia and after reperfusion.

incision as the preischemia group, though nothing was infused. Blood samples (about 0.5 ml) were taken from the thoracic inferior *vena cava* to determine the plasma glucose concentration. Animals in each group were sacrificed by exsanguination from the aorta before ischemia (preischemia), after 90 min of ischemia (ischemia), and after 60 min of reperfusion (reperfusion). Blood was collected and used to determine plasma insulin and lipid peroxide levels. Both the ischemic and non-ischemic lobes of the liver were used to prepare mitochondria.

Measurement of plasma levels of glucose and insulin. Plasma was separated from whole blood by centrifugation at $1,600 \times g$ for 20 min at room temperature. Glucose level in 50 μl of plasma was determined with a glucose electrode (Yellow Springs Instrument, Co., Inc., OH) to titrate the production of hydrogen peroxide by glucose oxidase in the buffer solution provided by the manufacturer (YSI 2357 Buffer Concentrate Kit).

Plasma insulin was estimated by use of a radioimmunoassay kit (INCSTAR Co., MN) which measured the extent of displacement of [^{125}I]-rat insulin standards by insulin in plasma samples.

Preparation of mitochondria. Liver mitochondria were isolated by the method of Schneider and Hogeboom [23] at 2-4°C. Briefly, liver was finely diced with scissors and homogenized with a glass homogenizer (Ten Broeck type) in 0.25 M sucrose, 1 mM ethylenediamine-N,N,N′,N′-tetraacetic acid (EDTA), and 20 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonate (Hepes) buffer (pH 7.4). The

homogenate was centrifuged at 600×*g* for 10 min, and the supernatant was centrifuged at 8,000×*g* for 10 min. The pellet was suspended in 0.25 M sucrose, 20 mM KCl, 20 mM Hepes buffer (pH 7.4) and was centrifuged again at 8,000×*g* for 10 min. The resultant pellet was suspended in 0.25 M sucrose, 20 mM KCl, 20 mM Hepes buffer (pH 7.4) and used as the mitochondrial preparation. Mitochondrial respiratory functions were determined immediately after the preparation.

Determination of α-tocopherol content. A mitochondrial suspension (1 ml) was mixed with 1 ml of an ethanol solution of 6% pyrogallol and 2 μg/ml tocol as an internal standard. The mixture was saponified at 70°C for 30 min after addition of 1/15 volume of 60% KOH. α-Tocopherol was extracted into *n*-hexane and was subjected to high-performance liquid chromatography (Hitachi 655α-11, Hitachi Industries, Tokyo) with a chromatograph equipped with a YMC-PAC α-600(NH_2) column (Yamamura Chemicals, Tokyo). A mixture of *n*-hexane and isopropanol (1 : 1, v/v) was used as the mobile phase. α-Tocopherol was estimated by fluorometry (excitation at 290 nm; emission at 325 nm) with a Hitachi F-1000 spectrofluorometer (Hitachi Industries).

Assay of mitochondrial respiratory functions. Respiratory function of mitochondria in the presence of succinate as a substrate was determined by measuring oxygen consumption with an oxygen probe (Yellow Springs Instrument, Co., Inc.) at 25°C. The reaction was started by the sequential addition of 50 μl mitochondrial preparation (30–50 mg/ml protein), 50 μl of 100 mM succinate (pH 7.4), and then 5 μl of 48.2 mM ADP to 1.75 ml of a medium containing 0.25 M sucrose, 20 mM KCl, 5 mM $MgCl_2$, 10 mM potassium phosphate buffer (pH 7.4), and 20 mM Hepes buffer (pH 7.4), which had been equilibrated by aeration for at least 1 h at 25°C. The inital oxygen concentration in the medium was assumed to be 245 μM. The respiratory control index (RCI) was calculated by dividing the rate of oxygen consumption in the presence of ADP (state 3) by the rate in the absence of ADP (state 4). The phosphate-to-oxygen ratio (P/O) was calculated by dividing the amount of consumed ADP by the amount of oxygen consumed in state 3. The oxidative phosphorylation rate (OPR) was calculated by multiplying the state 3 rate by P/O. The ATP synthesis rate, which was equal to OPR per g wet tissue, was the product of the OPR and the mitochondrial yield.

Assay of cytochrome c oxidase (CCO) activity. Activity of CCO was determined from the initial rate of disappearance of reduced cytochrome *c* by spectrophotometric titration at 550 nm in 0.1% Tween 80 and 0.1 M potassium phosphate buffer (pH 7.0) at 37°C. The extinction coefficient of reduced cytochrome *c* was assumed to be 22.1 $mM^{-1} \cdot cm^{-1}$.

Determination of lipid peroxides in mitochondria and plasma. Lipid peroxide levels in mitochondria or plasma was estimated as malondialdehyde by measuring thiobarbituric acid-reactive substances (TBARS) formed after incubating plasma [24] and mitochondria [25] with thiobarbituric acid. A slight modification was made for measuring lipid peroxides formed in mitochondria. Mitochondria were washed with 154 mM KCl and were centrifuged at 8,000×*g* for

10 min to remove sucrose, which may interfere with the thiobarbituric acid reaction. The resultant pellet was suspended in 154 mM KCl (30–50 mg/ml protein). The mitochondrial suspension (50 μl) was mixed with 50 μl of 8.1% (w/v) sodium dodecylsulfate, followed by the addition of 0.75 ml of distilled water and 0.5 ml of 20% (w/v) sodium acetate (pH 3.5). After the addition of 0.5 ml of 0.8% (w/v) thiobarbituric acid with mixing, the mixture was heated at 95°C for 60 min and was immediately chilled with tap water. Tetraethoxypropane was used as a standard to estimate the malondialdehyde formed in samples. Samples were diluted with physiologic saline to give an absorbance below 0.3 at the time of measurement.

Others. α-Tocopherol and the vehicle were donated by Eisai Co., Ltd. (Tokyo). Sodium pentobarbitone was obtained from Abbot Laboratories (IL); heparin sulfate, from NOVA Industry A/S (Bagsvaerd, Denmark); cytochrome *c*, from Sigma Chemical Co. (MO); and ADP, from Kohjin Chemicals (Tokyo). Hepes and EDTA came from Dojindo Laboratories (Kumamoto, Japan). All other reagents used were of analytical grade and purchased from either Wako Pure Chemicals (Osaka, Japan) or Nacalai Tesque (Kyoto, Japan).

Protein concentration was determined by the method of Lowry *et al.* [26] with bovine serum albumin used as a standard. Concentration of ADP was determined spectrophotometrically (Cary 2290, Victoria, Australia) at 259 nm with 15.4 $mM^{-1} \cdot cm^{-1}$ used as the extinction coefficient.

Statistical analysis. All results were expressed as the mean $\pm$ standard deviation. Significance of differences between values within each group (α-tocopherol and control group) was assessed by analysis of variance, followed by Scheffe's F-test. Significance of differences in values between the α-tocopherol group and the control group under similar conditions was analyzed by Student's *t*-test. Differences were considered significant when the *p* value was lower than 0.05.

RESULTS

Levels of glucose and insulin in plasma

Levels of glucose and insulin in plasma taken from the α-tocopherol and the control groups were determined before ischemia (preischemia), at the end of ischemia (ischemia), and after reperfusion (reperfusion; Table 1). Sham operation caused no marked difference in plasma glucose level between control (average at 1.35 mg/ml, $n=3$) and α-tocopherol group (average at 1.42 mg/ml, $n=3$). Plasma glucose levels remained relatively constant in both the control and α-tocopherol groups before and after ischemia, even with the administration of glucose. On the other hand, plasma glucose levels after reperfusion were significantly higher than preischemic and ischemic levels in both groups, and the level in the α-tocopherol group was significantly lower than that in the control group.

No marked difference in the plasma insulin was found in sham group between control (average at 4.0 ng/ml, $n=3$) and α-tocopherol groups (average at 3.8 ng/

ml, $n=3$). However, the level in the control group tended to be decreased in the ischemia group compared with the preischemia value and was significantly increased in the reperfusion group compared with the preischemia and the ischemia values. Similar findings were obtained in α-tocopherol group, although the differences were statistically insignificant.

Content of α-tocopherol in mitochondrial fractions

Table 2 shows the α-tocopherol content in mitochondria isolated from the ischemic and non-ischemic lobes of control and α-tocopherol groups in preischemia, ischemia, and reperfusion groups. Mitochondria in the α-tocopherol group accumulated significantly more (2.2–2.4 times) α-tocopherol than those in the control group before ischemia, showing that administered α-tocopherol could reach the mitochondria in the liver. The mitochondrial α-tocopherol content in ischemic lobes was significantly decreased in the ischemia group and further in the reperfusion group in both the control and α-tocopherol groups, although no statistically significant change occurred during reperfusion. In the non-ischemic lobes, the α-tocopherol content remained unchanged during ischemia and subsequent reperfusion in both groups.

Table 1. Plasma levels of glucose and insulin before ischemia, after ischemia, and after reperfusion (mean±SD).

		Control	(*n*)	α-Tocopherol	(*n*)
Glucose level (mg/ml plasma)	Preischemia	2.04±0.58	(6)	2.09±0.44	(6)
	Ischemia	2.39±0.93	(5)	1.84±0.44	(5)
	Reperfusion	5.64±0.89‡‡§§	(11)	4.29±1.37*,‡‡§§	(11)
Insulin level (ng/ml plasma)	Preischemia	6.1 ±1.9	(6)	6.2 ±2.7	(6)
	Ischemia	4.2 ±2.7	(5)	3.9 ±1.3	(5)
	Reperfusion	10.7 ±3.1‡§§	(6)	7.8 ±2.9	(7)

*Control vs. α-tocopherol ($p<0.05$); ‡preischemia vs. reperfusion (‡$p<0.05$; ‡‡$p<0.01$); §§ischemia vs. reperfusion ($p<0.01$).

Table 2. Content of α-tocopherol (ng/mg mitochondrial protein, mean±SD) in the mitochondrial preparations.

	Control	(*n*)	α-Tocopherol	(*n*)
Ischemic lobes				
Preischemia	193±34	(6)	429±61	(6)
Ischemia	129±19††	(5)	298±50†	(5)
Reperfusion	96±11‡‡	(8)	268±71‡‡	(8)
Non-ischemic lobes				
Preischemia	171±34	(6)	414±52	(6)
Ischemia	160±26	(5)	416±114	(5)
Reperfusion	192±36	(8)	404±80	(8)

†Preischemia vs. ischemia (†$p<0.05$; ††$p<0.01$); ‡preischemia vs. reperfusion ($p<0.01$). α-Tocopherol content in the α-tocopherol group was significantly higher than the control level ($p<0.01$) under any condition in both ischemic and non-ischemic lobes.

Table 3. Yields of mitochondrial protein (mg mitochondrial protein/g wet tissue, mean ± SD).

	Control	(*n*)	α-Tocopherol	(*n*)
Ischemic lobes				
Preischemia	17.1±2.8	(6)	16.2±2.3	(6)
Ischemia	15.7±1.2	(5)	16.1±1.6	(5)
Reperfusion	13.8±1.1‡	(8)	14.9±1.2	(8)
Non-ischemic lobes				
Preischemia	16.2±1.8	(6)	17.1±2.4	(6)
Ischemia	16.3±2.4	(5)	15.7±1.4	(5)
Reperfusion	16.7±1.0	(8)	16.9±1.7	(8)

‡Preischemia vs. reperfusion ($p<0.05$).

Yields of mitochondrial protein

Efficiency of mitochondrial isolation was assessed in terms of protein yield in the mitochondrial preparations (Table 3). In ischemic lobes, ischemia slightly lowered the yield of mitochondrial protein in the control group, whereas no apparent difference in the yield was observed in the α-tocopherol group at the end of ischemia. Reperfusion significantly decreased the yield in the control group, whereas the decrease in the α-tocopherol group was not significant. In the non-ischemic lobes, no significant difference was found in either group.

Mitochondrial respiratory function

Respiratory functions of mitochondria isolated from ischemic lobes (Fig. 2) and non-ischemic lobes (Fig. 3) of control and α-tocopherol groups were examined in preischemia, ischemia, and reperfusion groups of livers. Respiratory functions in either ischemic or non-ischemic lobes from the preischemia group were not significantly different from those in the sham-operated liver (data not shown). Therefore, it is unlikely that the first glucose infusion affected mitochondrial function.

State 3 respiration in ischemic lobes (Fig. 2A) was significantly decelerated during ischemia in both control and α-tocopherol groups. However, reperfusion significantly accelerated state 3 respiration in both groups, although the rates were still significantly lower than those of the preischemia group. Recovery of state 3 rate after reperfusion was significantly higher in the α-tocopherol group than in the control group. On the other hand, state 4 respiration was significantly accelerated during ischemia in both control and α-tocopherol groups (Fig. 2B). Reperfusion restored the rate to the preischemic level in both groups, and α-tocopherol significantly reduced the rate that had been increased during ischemia. RCI (Fig. 2C), P/O (Fig. 2D), and OPR (Fig. 2E) showed profiles similar to the profile for the state 3 respiration rate: They were significantly lowered during ischemia and showed tendency to be restored during reperfusion in both of control and α-tocopherol groups. Values after reperfusion were significantly higher in the α-tocopherol group compared with those for the control group. Taking mitochon-

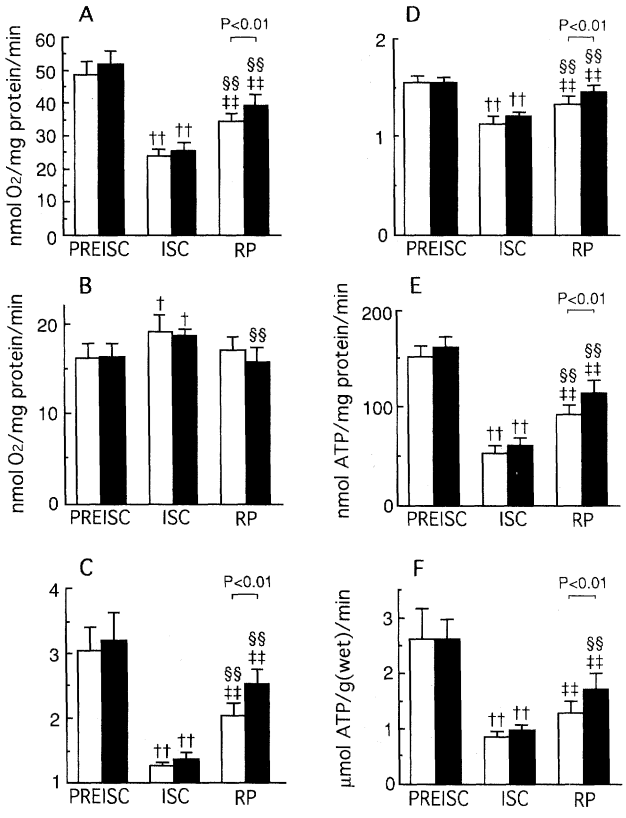

Fig. 2. Mitochondrial respiratory functions in ischemic lobes are compared between control (open columns) and α-tocopherol (filled columns) groups. Mitochondria were isolated from preischemic (PREISC, $n=6$), ischemic (ISC, $n=5$), and reperfused (RP, $n=8$) livers. (A), State 3 rate; (B), state 4 rate; (C), respiratory control index; (D), phosphate-to-oxygen ratio; (E), oxidative phosphorylation rate; (F), ATP synthesis rate. No significant difference was observed between sham group (data not shown) and preischemic group. Bars indicate standard deviation. †Preischemic vs. ischemic groups; ‡preischemic vs. reperfused groups; §ischemic vs. reperfused groups. $^{\dagger}p<0.05$; ††,‡‡ and $^{\S\S}p<0.01$. Significant difference between control and α-tocopherol groups is indicated over columns in the figure, when applicable.

drial yields (Table 3) into account, the ATP synthesis rate per unit weight was calculated (Fig. 2F). The rate was significantly reduced in both control and α-tocopherol groups during ischemia. Although the rate was increased in both groups after reperfusion, only the α-tocopherol group showed a significant increment from the ischemic level.

In non-ischemic lobes (Fig. 3), state 3 respiration was significantly accelerated in ischemia and reperfusion groups of both control and α-tocopherol treated animals (Fig. 3A). No statistically significant difference was shown in state 4

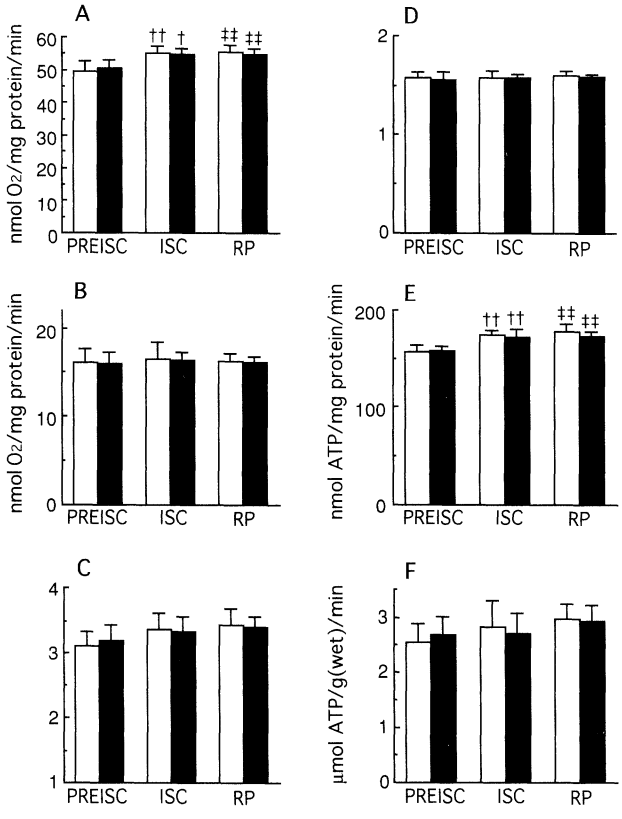

Fig. 3. Mitochondrial respiratory functions in non-ischemic lobes are compared between control (open columns) and α-tocopherol (filled columns) groups. Mitochondria were isolated from preischemic (PREISC, $n=6$), ischemic (ISC, $n=5$), and reperfused (RP, $n=8$) livers. (A), State 3 rate; (B), state 4 rate; (C), respiratory control index; (D), phosphate-to-oxygen ratio; (E), oxidative phosphorylation rate; (F), ATP synthesis rate. No significant difference was observed between sham group (data not shown) and preischemia group. Bars indicate standard deviation. †Preischemic vs. ischemic groups; ‡preischemic vs. reperfused groups. †$p<0.05$; †† and ‡‡$p<0.01$. There was no significant difference between control and α-tocopherol groups in any function.

respiration, RCI, or P/O in either treatment group before or at the end of ischemia or after reperfusion (Fig. 3B, C, and D). On the other hand, OPR was significantly accelerated during ischemia in both control and α-tocopherol groups, though subsequent reperfusion treatment did not affect the rate, which remained the same as in the ischemia group (Fig. 3E). In contrast to ischemic lobes, non-ischemic lobes showed no statistically significant change in the ATP synthesis rate following ischemia or reperfusion in either the control or α-tocopherol group (Fig. 3F).

CCO activity

The activity of CCO in ischemic-lobe mitochondria decreased in the ischemia group by 35 and 30% in control and α-tocopherol groups, respectively (Table 4). Reperfusion apparently increased the activity that had been suppressed during ischemia, in both the control and α-tocopherol groups, between which no significant difference was found. The activity in the reperfusion group stayed significantly low only in the control group. The activities in mitochondria from non-ischemic lobes were similar between preischemia, ischemia, and reperfusion groups and between the control and α-tocopherol groups.

Lipid peroxide levels in plasma and mitochondria

The content of plasma lipid peroxides was estimated in plasma samples from control and α-tocopherol treated animals of preischemia, ischemia, and reper-

Table 4. Cytochrome *c* oxidase activity (nmol/mg mitochondrial protein/min, mean ± SD) in mitochondrial preparations.

	Control	(*n*)	α-Tocopherol	(*n*)
Ischemic lobes				
Preischemia	448±35	(6)	437±68	(6)
Ischemia	291±41††	(5)	305±59††	(5)
Reperfusion	348±50‡‡	(8)	387±55	(8)
Non-ischemic lobes				
Preischemia	448±38	(6)	450±27	(6)
Ischemia	470±30	(5)	466±50	(5)
Reperfusion	472±30	(8)	467±27	(8)

††Preischemia vs. ischemia ($p<0.01$); ‡‡preischemia vs. reperfusion ($p<0.01$).

Table 5. The lipid peroxide level in plasma and mitochondria before ischemia, after ischemia, and after reperfusion.

	Control	(*n*)	α-Tocopherol	(*n*)
Plasma (nmol/ml)				
Preischemia	12.6±2.9	(6)	10.5±3.3	(6)
Ischemia	10.0±1.1	(5)	8.2±1.8	(5)
Reperfusion	18.3±4.8‡§§	(11)	11.4±2.6**	(11)
Mitochondria (nmol/mg protein)				
Ischemic lobes				
Preischemia	2.00±0.45	(6)	1.97±0.39	(6)
Ischemia	2.44±0.28	(5)	2.28±0.39	(5)
Reperfusion	2.90±0.29‡‡	(8)	2.30±0.23**	(8)
Non-ischemic lobes				
Preischemia	1.98±0.48	(6)	1.80±0.37	(6)
Ischemia	1.98±0.25	(5)	1.85±0.33	(5)
Reperfusion	1.86±0.21	(8)	1.88±0.32	(8)

Levels of lipid peroxides were estimated as TBARS. **Control vs. α-tocopherol ($p<0.01$); ‡preischemia vs. reperfusion (‡$p<0.05$; ‡‡$p<0.01$); §§ischemia vs. reperfusion ($p<0.01$).

fusion groups (Table 5). The plasma lipid peroxide level apparently decreased in both control and α-tocopherol groups during ischemia, whereas the level in the reperfusion group was significantly higher compared with both preischemic and ischemic levels in the control group. On the other hand, there was no statistically significant difference in the plasma lipid peroxide level in α-tocopherol group. The elevated plasma level in the reperfusion group was significantly higher in the control group than in the α-tocopherol group. We also estimated the lipid peroxide level in mitochondrial preparations that were isolated from the ischemic and non-ischemic lobes of control and α-tocopherol groups before and at the end of ischemia and after reperfusion (Table 5). In ischemic lobes of the control group, the level was apparently increased in the ischemia group and significantly so in the reperfusion group. In contrast, the level in the α-tocopherol group was slightly increased in ischemia group, and no further increase was found in the reperfusion group. There was no significant difference between control and α-tocopherol groups of preischemic or ischemic livers, whereas the increase in the level after reperfusion was significantly suppressed by administration of α-tocopherol. The levels remained unchanged in non-ischemic lobes in both control and α-tocopherol groups during ischemia and subsequent reperfusion.

DISCUSSION

We showed in the present study that α-tocopherol, as a radical scavenger, suppressed elevation of blood glucose and damage in hepatic mitochondria during ischemia and reperfusion of liver, while glucose was being intravascularly supplemented. Plasma glucose is known to be elevated when an excess of glucose is taken while metabolic functions of the liver are severely disturbed [1]. It is therefore important to design nutritional support after an operation to avoid elevation of blood glucose. A close relationship between glucose metabolism and mitochondrial functions has been observed in liver cells [2, 27]. Mitochondrial damage has recently been pointed out to be responsible for ischemia-reperfusion injury of various organs including liver [9–11, 13–15]. Furthermore, superoxides have been blamed for the mitochondrial damage during ischemia and reperfusion [13, 14]. α-Tocopherol was therefore expected to protect mitochondria from ischemia-reperfusion injury and to subsequently attenuate elevation of plasma glucose level when glucose was given. Since glucose was intermittently infused to evaluate glucose tolerance in the present study, plasma glucose levels were different between the preischemia, ischemia, and reperfusion groups. However, examination of mitochondrial function in the non-ischemic lobes of liver showed the absence of such a functional deviation due to different plasma glucose levels. Different plasma glucose levels would thus not affect mitochondrial function in the present study. The difference in the plasma glucose levels between control and α-tocopherol groups after reperfusion was not likely due to lowered plasma insulin level, since control plasma after reperfusion showed significantly higher glucose and

apparently higher insulin levels than the plasma of the α-tocopherol group. Hyperglycemia is often found in patients with chronic liver diseases associated with impaired glucose uptake in skeletal muscles [28], whereas impaired glucose tolerance occurs within a short period after resection and transplantation of normal liver in a manner depending on the extent of invasion. Significant elevation of plasma glucose in the control group compared with the level for the α-tocopherol group at the early phase of reperfusion might be attributed to the severe liver damage, though we did not examine the sensitivity of skeletal muscles to insulin. Besides facilitation of glucose permeation across plasmalemma of skeletal muscle cells, insulin regulates activities of various enzymes that participate in glucose metabolism in liver cells, in which ATP and NADH play important roles [29]. We therefore attempted to assess liver function by examining oxidative phosphorylation of mitochondria isolated from ischemic and non-ischemic lobes of liver at the end of ischemia and reperfusion to evaluate mitochondrial activity, which should control cellular levels of ATP and NADH.

Mitochondrial yield is likely to be lowered when cells and/or mitochondria disintegrate as is the case with myocytolysis during ischemia and reperfusion of the myocardium [30, 31]. Such structural damage might contribute to lowered mitochondrial yield after reperfusion of control ischemic lobes in the present study. On the other hand, administration of α-tocopherol suppressed the decrease in mitochondrial yield after reperfusion of ischemic lobes. These results may suggest that α-tocopherol accumulated in mitochondria of the α-tocopherol group contributed to prevent structural damage, which damage could result in lowered mitochondrial yield as seen in the control group.

Phosphorylating activities of mitochondria were significantly lowered during ischemia in both control and α-tocopherol groups in ischemic lobes. Those values were increased after reperfusion and the increment was much larger in the α-tocopherol group than in the control group. α-Tocopherol therefore could protect mitochondrial respiration from damage during reperfusion. In contrast to ischemic lobes, non-ischemic lobes showed an enhanced OPR during ischemia and reperfusion in both control and α-tocopherol groups. This result was coincident with the findings of Wang *et al.* [21], who indicated that an increased OPR in non-ischemic lobes at the end of ischemia resembled a phenomenon seen in major partial hepatectomy. However, the increase in OPR after reperfusion in the present study was not so marked as they reported. The discrepancy between their findings and ours may be due to the difference in plasma glucose level, the extent of ischemia (50% vs. 70%), and the substrate used for analysis of mitochondrial functions (glutamate vs. succinate). ATP synthesis rates after ischemia and after reperfusion were decreased in ischemic lobes, whereas they were increased in non-ischemic lobes of both groups. Non-ischemic lobes were therefore likely to compensate for the suppressed functions in ischemic lobes in terms of energy supply. CCO has been reported to be tolerant to various stresses [17–19]. In the present study, however, CCO activity in ischemic lobes was lowered during

ischemia and considerably restored by reperfusion, which was similar to changes seen in the respiratory functions. However, the OPR was lessened by ischemia and reperfusion more severely than CCO activity in both control and α-tocopherol groups. This fact may indicate that the mitochondrial dysfunction described above would occur at the upstream of complex IV in both groups, which dysfunction was lessened by α-tocopherol.

Active oxygen species have been suggested to be responsible for ischemia-reperfusion injury of liver [9–15, 32]. Free radical scavengers such as superoxide dismutase [32–35], catalase [35], and allopurinol [22, 34, 36] have succeeded to reduce reperfusion injury in various organs. α-Tocopherol has also been reported to show a protective effect against active oxygen species in liver [10, 37, 38]. Since lipid peroxidation inactivates various membrane-associated enzymes [39–42], an increase in lipid peroxides in mitochondrial membranes, which have an abundance of unsaturated fatty acid moieties [43], could contribute to a decrease in mitochondrial activity. Preischemic lipid peroxide levels in mitochondrial fractions of ischemic lobes were similar between control and α-tocopherol groups. The level in the control group increased during ischemia and reperfusion, whereas the increase was not marked during reperfusion in the α-tocopherol group. On the other hand, mitochondrial α-tocopherol decreased more markedly during ischemia than during reperfusion. Therefore, it is likely that α-tocopherol could be effective to lower the lipid peroxide level during reperfusion by suppressing formation of free radicals during ischemia. These findings suggest that there exists a close relationship between respiratory function and lipid peroxidation of mitochondria and that administered α-tocopherol effectively protects mitochondria against oxidative damage.

The present study indicates that impaired glucose tolerance in the early phase of reperfusion with infusion of glucose was unlikely due to lowered plasma insulin level. Mitochondrial damage in ischemic lobes during reperfusion occurs mainly before complex IV, to which lipid peroxidation might contribute. Pretreatment with α-tocopherol suppressed both of the above phenomena. Oxidative phosphorylation in non-ischemic lobes is likely to compensate for suppressed mitochondrial activity in the ischemic lobes during ischemia and reperfusion. In conclusion, functional damage in liver mitochondria is, at least in part, likely to contribute to glucose intolerance in the early phase of reperfusion. Administration of α-tocopherol may thus be effective in clinical cases to avoid hyperglycemia and liver injury due to reperfusion following ischemia and to lessen the amount of insulin required.

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