Suspected Myotoxicity of Edible Wild Mushrooms

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Recently, the widely consumed yellow tricholoma *Tricholoma flavovirens* caused delayed rhabdomyolysis and fatalities in humans in France and Poland and triggered elevated plasma creatine kinase activities in mice. Furthermore, the highly appreciated king boletus (*Boletus edulis*) caused similar responses in experimental mice. Because of this, it was hypothesized that other fungi could also contain chemical compounds that would cause similar myotoxic effects. To test the suspected myotoxicity of other wild mushrooms consumed by tradition, 86 mice were exposed for 5 days to 3, 6, or 9 g/kg body mass/day of edible mushrooms representing diverse genera (*Russula* spp, *Cantharellus cibarius*, *Albatrellus ovinus*, and *Leccinium versipelle*) mixed with regular laboratory rodent diet. The plasma creatine kinase activity increased with all studied mushroom species at 9 g/kg body mass/day, whereas the histologic appearance of muscle and liver samples was unaffected. The results support the hypothesis that the previously observed toxic effects are not specific to *T. flavovirens*, but probably represent an unspecific response requiring individual sensitivity and a significant amount of ingested mushroom to manifest itself. Exp Biol Med 231:221–228, 2006

Key words: *Albatrellus ovinus*; *Cantharellus cibarius*; creatine kinase; *Leccinium versipelle*; rhabdomyolysis; *Russula* spp

Introduction

The consumption of wild mushrooms is widespread in Europe and North America and the choice of species for consumption is mostly dictated by tradition. Recently, it was reported in France and Poland that several people developed delayed and, in some cases, fatal rhabdomyolysis after consuming large amounts of the yellow tricholoma (*Tricholoma equestre* or *Tricholoma flavovirens*) during several consecutive meals (1–3). Furthermore, it was shown in mice that 4–6 g of powdered *T. flavovirens* per kilogram per day increased plasma creatine kinase (CK) activities and caused tachypnea, reduced motor activity, diarrhea, and muscle fiber disorganization visible in light microscopy (1). This was confirmed later by another study demonstrating the same effect of increased plasma CK activities at 9 g *T. flavovirens*/kg/day (4). In addition, it was discovered that the same response can be triggered by the king boletus (*Boletus edulis*) consumed widely and considered delicious in Europe and Alaska (5). In addition, the Asian *Russula subnigricans* has been a causative agent of rhabdomyolysis in nine Taiwanese cases (6).

Because of the established tradition of consuming wild mushrooms and marketing them, e.g., in North European marketplaces and French supermarkets, the subject of myotoxicity is of urgent public importance. The aim of this study was to start a screening project on widely consumed mushrooms to establish (i) whether elevated CK activities can be demonstrated by ingestion of other mushroom species and genera; (ii) what the dose required for the effects to manifest would be; and (iii) what other possible effects on basic biochemical variables reflecting the health and well-being of experimental animals can be demonstrated resulting from mushroom ingestion. The mushroom species chosen for this study represent different genera with a long tradition of human consumption. The chanterelle (*Cantharellus cibarius*, Cantharellaceae) is a species sought after and sold commercially. The different edible Russula species (*R. xerampelina*, *R. flava*, *R. vinosa*, and *R. decolorans*), belonging to the same Russulaceae family as *R. subnigricans*, the species with previous reports of rhabdomyolysis (6–8), are brightly colored and easily recognizable species, usually collected and cooked in a mixture containing several russulas. The brown birch boletus (*Leccinium versipelle*) is a typical member of the Boletaceae family, causing mild stomach irritation unless properly cooked (9). Finally, the sheep polypore (*Albatrellus ovinus*) is of the Polyporaceae family and can be consumed fresh or dried.

Materials and Methods

The different mushrooms (Fig. 1a–g) were harvested in a mixed Picea and deciduous forest in the small islands of

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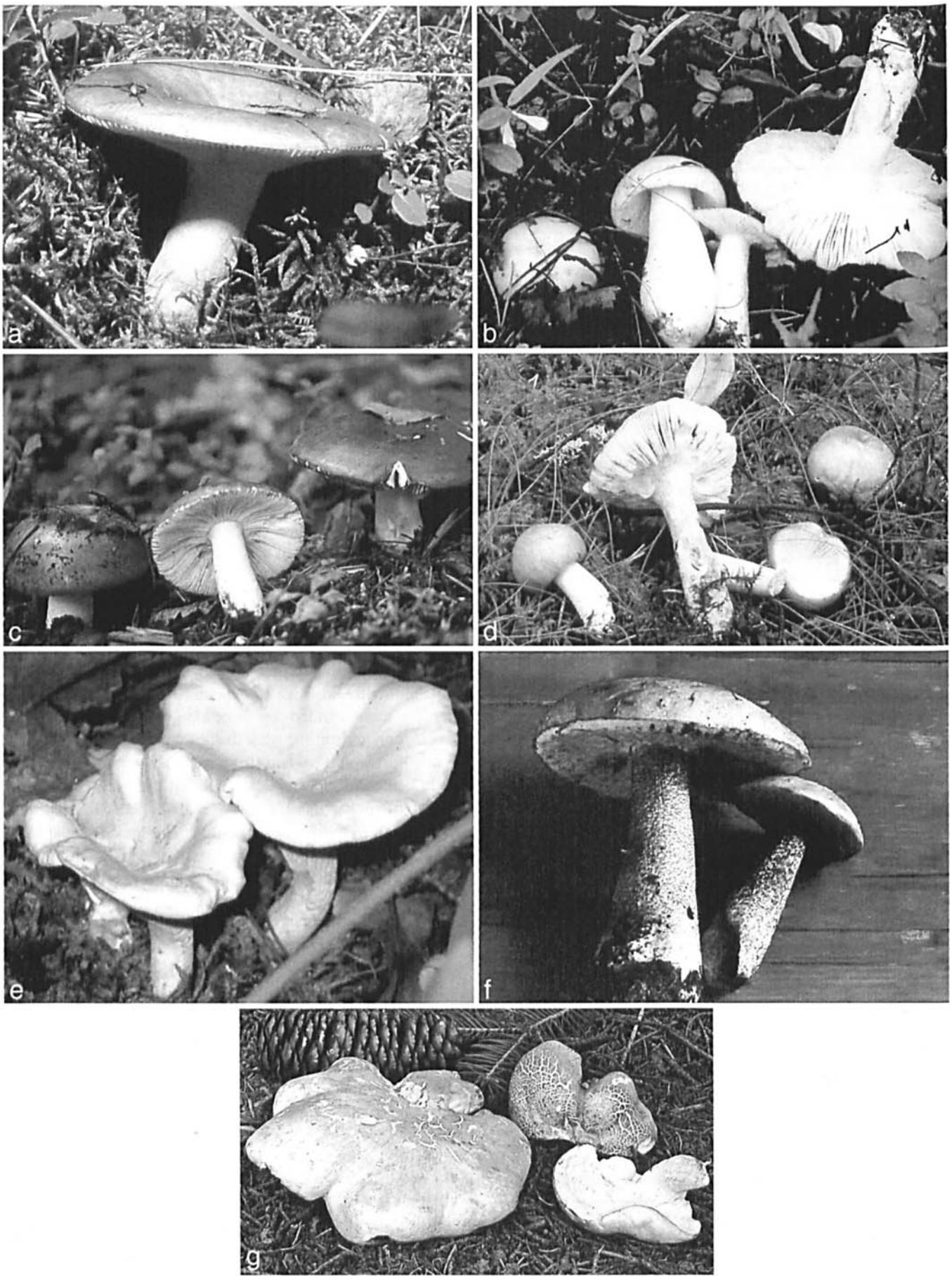
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Toivonsaari and Vuorisaari of the Lake Haukivesi, Linnansaari National Park, Finland (69°00′N 35°70′E) in September 2003. The island is distant (at least 20 km) from any road traffic or industrial establishment and, thus, significant amounts of heavy metals or other contamination would have been very unlikely in the fungi. In addition, some of the *C. cibarius* were collected in young, desolate Picea forest in the Liperi commune, in eastern Finland (69°40′N, 36°20′E). The identification of the species (10) was carried out using both taxonomic and microscopic characteristics (spores and basidia) by the botanical specialist of the research team (M. Kirsi), and some of the dried specimens were stored at the University of Joensuu for later confirmation. The fungi were weighed and dried, pulverized, and mixed into the feed of the experimental animals.

The experimental animals, 86 female laboratory mice (*Mus musculus*; age 44–113 days) of the breeding colony of the University of Joensuu, were housed in groups of 2–4 individuals in standard wire cages (Makrolon: 42 × 22 × 15 cm) with wood shavings for bedding and free access to water and a pelleted diet (Avelsfoder för råtta och mus R36: carbohydrates 55.7%, protein 18.5%, fat 4.0%, and energy content 1260 kJ/100 g; Lactamin, Stockholm, Sweden). At the beginning of the study, the animals were divided into 14 experimental groups as follows. Group 1: control group receiving regular feed; Group 2: positive control group receiving 75 mg/kg body mass (BM)/day *p*-phenylenediamine (catalog #P6001-50G; Sigma Chemical Co., St. Louis, MO) known to cause rhabdomyolysis in mice (1, 4); Groups 3–14 were the experimental mushroom groups receiving 3, 6, or 9 g of dried *C. cibarius*, *Russula* spp, *L. versipelle*, or *A. ovinus* powder per kilogram BM per day (18, 36, or 54 g dried mushroom/kg feed/day; based on previous measurements of food intake of mice with a similar BM). The pelleted food was moisturized into a homogenous mass, into which the mushroom powders or the dissolved *p*-phenylenediamine were added. The food was then mixed carefully, dried, and pelleted again. All of the feeding regimes lasted for 5 days. The animals were given food and water *ad libitum*. The mice were weighed at the beginning of the study and at sampling. Food intake was measured by weighing the amount of food left uneaten. After 5 days of exposure, the animals were euthanized with diethyl ether. Their BM and length were measured. Blood samples were obtained with cardiac punctures with sterile needles and syringes, transferred into test tubes containing EDTA, and centrifuged at 4000 *g* to obtain plasma. The livers and kidneys were dissected and weighed. Muscle samples were taken from the quadriceps muscle of the left hind limb. The plasma samples were frozen in liquid nitrogen and stored at −70°C. The muscle and liver samples were stored in formalin. For the histologic analyses, the liver and muscle samples were dehydrated and embedded in paraffin. The samples were cut into 8- to 10-μm sections and attached to glass slides for staining with hematoxylin-eosin. The possible changes in histologic appearance were recorded under a light microscope.

The plasma total cholesterol was determined with the cholesterol enzymatic end point method of Randox Laboratories Ltd. (Crumlin, UK). The plasma low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol levels were measured with the direct LDL- and HDL-cholesterol reagents of Randox Laboratories. Plasma triacylglycerol and glucose levels were measured with the GPO-PAP and liquid reagent hexokinase methods, and the creatinine concentrations were determined with the creatinine colorimetric method (Randox Laboratories). Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured with the ALT (GPT) Alanine Aminotransferase EC 2.6.1.2 European Committee on Clinical Laboratory Standards (ECCLS) and AST (GOT) Aspartate Aminotransferase EC 2.6.1.2 ECCLS reagents, and the total CK activities were determined with the CK NAC-activated Creatine Kinase EC 2.7.3.2 reagents (Randox Laboratories). For the actual measurements, the Technicon RA-XT Analyser (Swords, Ireland) was used.

The normality of distribution and the homogeneity of variances were tested with the Kolmogorov-Smirnov test and the Levene test. Comparisons between the study groups were performed with the one-way analysis of variance (ANOVA) and the *post hoc* Dunnett's test or, if homogeneity of variances and normal distribution were not attained after standard transformations, with the Kruskal-Wallis test. The $P < 0.05$ level was considered statistically significant.

Results

All experimental animals remained in good health until the end of the experiment and no specific symptoms, such as diarrhea, were present during the study. The actual amounts of the experimental substances consumed by the mice varied slightly from the theoretical values (Tables 1 and 2). However, they were very close to the doses of the study design.

Russula spp at 6 g/kg/day caused a reduction in the BM of the animals compared with the control mice (Table 1), whereas the mice fed with *A. ovinus* at 3 g/kg/day gained more weight than the control mice (Table 2). The absolute food intake of the *p*-phenylenediamine–treated mice was lower than in the control mice, whereas the absolute water intake was higher in the *p*-phenylenediamine–treated group compared with the control group (Tables 1 and 2). In addition, the relative water intake was higher in two of the

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Figure 1. (a) *R. xerampelina*, (b) *R. flava*, (c) *R. vinosa*, (d) *R. decolorans*, (e) *C. cibarius*, (f) *L. versipelle*, and (g) *A. ovinus* (pictures taken by the authors).

Table 1. Body Mass, Food Intake, Water Intake, Organ Weights, and Plasma Biochemical Variables of the Mice Fed with *Russula* spp or *C. cibarius* (Mean ± SE)[a]

	Control	*p*-Phenylene-diamine (75 mg/kg/d)	Dried *Russula* spp (3 g/kg/d)	Dried *Russula* spp (6 g/kg/d)	Dried *Russula* spp (9 g/kg/d)	Dried *C. cibarius* (3 g/kg/d)	Dried *C. cibarius* (6 g/kg/d)	Dried *C. cibarius* (9 g/kg/d)
Amount ingested (g/kg/d)	—	88 ± 0.8 (mg)	2.8 ± 0.1	5.3 ± 0.3	8.4 ± 0.2	3.2 ± 0.1	6.1 ± 0.1	9.3 ± 0.2
Equivalent dose of fresh mushroom (g/kg)	—	—	19.3	36.5	57.9	22.1	42.1	64.1
Equivalent dose of fresh mushroom for a 70-kg person (kg/d)	—	—	1.35	2.56	4.06	1.54	2.94	4.49
BM start (g)	25.1 ± 0.5	24.1 ± 0.5	24.7 ± 1.1	22.4 ± 0.5	23.8 ± 0.5	24.0 ± 0.6	24.5 ± 0.7	24.9 ± 0.6
BM end (g)	25.4 ± 0.6	25.3 ± 0.8	23.9 ± 0.7	21.6 ± 0.6	23.5 ± 0.7	24.5 ± 0.7	24.6 ± 0.8	25.1 ± 0.7
BM change (g)	+0.3 ± 0.2	+1.2 ± 0.6	−0.8 ± 0.6	−0.9 ± 0.1***	−0.4 ± 1.0	+0.5 ± 0.3	0.0 ± 0.2	+0.2 ± 0.4
Food intake (g)	24.0 ± 0.4	21.3 ± 0.3***	23.2 ± 0.4	22.2 ± 1.2	23.2 ± 0.5	26.2 ± 0.6	25.2 ± 0.3	25.9 ± 0.7
Food intake/BM (g)	0.95 ± 0.02	0.84 ± 0.02	0.97 ± 0.02	1.00 ± 0.03	1.02 ± 0.05	1.07 ± 0.02	1.03 ± 0.02*	1.03 ± 0.03
Water intake (ml)	25.1 ± 0.6	43.4 ± 2.8***	26.4 ± 1.2	20.7 ± 1.1**	25.1 ± 0.6	27.8 ± 0.8**	25.3 ± 0.9	32.0 ± 1.7***
Water intake (ml/BM)	0.95 ± 0.03	1.73 ± 0.15***	1.10 ± 0.04**	0.96 ± 0.05	1.37 ± 0.23**	1.13 ± 0.03**	1.04 ± 0.07	1.28 ± 0.10**
Liver mass (mg)	1338 ± 45	1364 ± 28	1236 ± 28	1139 ± 41	1162 ± 75	1369 ± 60	1379 ± 84	1373 ± 61
Liver mass/BM (%)	5.3 ± 0.1	5.4 ± 0.2	5.2 ± 0.1	5.3 ± 0.2	4.9 ± 0.3	5.6 ± 0.2	5.6 ± 0.2	5.5 ± 0.2
Kidney mass (mg)	384 ± 11	372 ± 6	423 ± 48	342 ± 10	387 ± 20	370 ± 12	386 ± 18	400 ± 18
Kidney mass/BM (%)	1.51 ± 0.03	1.47 ± 0.03	1.77 ± 0.20	1.59 ± 0.03	1.65 ± 0.08	1.51 ± 0.05	1.57 ± 0.03	1.60 ± 0.07
Total cholesterol (mmol/L)	2.2 ± 0.1	1.8 ± 0.1**	2.3 ± 0.2	2.2 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.6 ± 0.2**	2.5 ± 0.2
LDL cholesterol (mmol/L)	0.18 ± 0.02	0.16 ± 0.01	0.15 ± 0.01	0.17 ± 0.02	0.14 ± 0.01	0.15 ± 0.01	0.18 ± 0.01	0.14 ± 0.01
HDL cholesterol (mmol/L)	1.35 ± 0.07	1.05 ± 0.03***	1.34 ± 0.04	1.27 ± 0.12	1.15 ± 0.07	1.37 ± 0.08	1.76 ± 0.12***	1.56 ± 0.18
HDL/total cholesterol	0.61 ± 0.01	0.59 ± 0.02	0.60 ± 0.03	0.58 ± 0.04	0.57 ± 0.02	0.65 ± 0.01*	0.68 ± 0.01**	0.63 ± 0.01
Triacylglycerols (mmol/L)	1.43 ± 0.08	1.22 ± 0.09	1.08 ± 0.09**	0.98 ± 0.08***	1.00 ± 0.08***	1.23 ± 0.12	1.21 ± 0.13	1.26 ± 0.08
Glucose (mmol/L)	13.1 ± 0.9	9.0 ± 1.9*	12.5 ± 0.8	11.0 ± 0.5	10.8 ± 0.6	10.8 ± 1.0	12.8 ± 0.5	12.6 ± 0.8
Creatinine (μmol/L)	57 ± 3	52 ± 4	64 ± 3	58 ± 4	44 ± 6	68 ± 7	80 ± 2***	65 ± 4
Creatine kinase (U/L)	212 ± 37	777 ± 157***	447 ± 142*	601 ± 252*	1334 ± 445***	625 ± 237*	195 ± 72	639 ± 182***
ALT (U/L)	67 ± 7	93 ± 13*	83 ±10	62 ± 7	82 ± 10	93 ± 33	43 ± 4**	52 ± 5
AST (U/L)	145 ± 18	239 ± 37**	230 ± 48*	183 ± 31	256 ± 62*	435 ± 182	156 ± 20	221 ± 38*

[a] Significant difference from the control group. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (one-way ANOVA).

Table 2. Body Mass, Food Intake, Water Intake, Organ Weights, and Biochemical Variables of the Mice Fed with *A. ovinus* or *L. versipelle* (Mean ± SE)[a]

	Control	*p*-Phenylene-diamine (75 mg/kg/d)	Dried *A. ovinus* (3 g/kg/d)	Dried *A. ovinus* (6 g/kg/d)	Dried *A. ovinus* (9 g/kg/d)	Dried *L. versipelle* (3 g/kg/d)	Dried *L. versipelle* (6 g/kg/d)	Dried *L. versipelle* (9 g/kg/d)
Amount ingested (g/kg/d)	—	88 ± 0.8 (mg)	3.0 ± 0.1	5.1 ± 0.2	8.6 ± 0.3	2.7 ± 0.1	5.2 ± 0.1	7.7 ± 0.1
Equivalent dose of fresh mushroom (g/kg)	—	—	20.7	32.1	59.3	18.6	35.9	53.1
Equivalent dose of fresh mushroom for a 70-kg person (kg/d)	—	—	1.45	2.46	4.15	1.30	2.51	3.71
BM start (g)	25.1 ± 0.5	24.1 ± 0.5	23.7 ± 0.3	23.2 ± 0.4	23.9 ± 0.2	20.7 ± 0.4	20.6 ± 0.4	20.2 ± 0.4
BM end (g)	25.4 ± 0.6	25.3 ± 0.8	25.0 ± 0.7	23.3 ± 0.8	25.0 ± 0.7	21.0 ± 0.5	20.5 ± 0.5	20.6 ± 0.3
BM change (g)	+0.3 ± 0.2	+1.2 ± 0.6***	+1.3 ± 0.2**	+0.1 ± 0.6	+1.0 ± 0.4	+0.3 ± 0.3	−0.1 ± 0.1	+0.5 ± 0.1
Food intake (g)	24.0 ± 0.4	21.3 ± 0.3***	24.8 ± 1.0	21.3 ± 0.9	23.9 ± 0.9	22.1 ± 0.3	21.7 ± 0.2	21.4 ± 0.5
Food intake/BM (g)	0.9 ± 0.02	0.8 ± 0.02***	1.0 ± 0.04	0.9 ± 0.04	1.0 ± 0.04	1.1 ± 0.02*	1.1 ± 0.03**	1.0 ± 0.02
Water intake (ml)	25.1 ± 0.6	43.4 ± 2.8***	26.0 ± 1.9	25.4 ± 1.6	26.8 ± 0.5	26.4 ± 0.2	26.2 ± 0.8	23.5 ± 0.6
Water intake (ml/BM)	0.95 ± 0.03	1.73 ± 0.15***	1.04 ± 0.07	1.09 ± 0.07	1.08 ± 0.02	1.26 ± 0.02	1.28 ± 0.05	1.14 ± 0.04*
Liver mass (mg)	1338 ± 45	1364 ± 28	1332 ± 27	1190 ± 22	1373 ± 38	1041 ± 83	1104 ± 25	1106 ± 44
Liver mass/BM (%)	5.3 ± 0.1	5.4 ± 0.2	5.3 ± 0.1	5.1 ± 01	5.5 ± 0.2	4.9 ± 0.3	5.4 ± 0.1	5.4 ± 0.1
Kidney mass (mg)	384 ± 11	372 ± 6	351 ± 18	350 ± 8	374 ± 10	318 ± 15	315 ± 11	319 ± 8
Kidney mass/BM (%)	1.51 ± 0.03	1.47 ± 0.03	1.40 ± 0.06	1.50 ± 0.04	1.50 ± 0.05	1.52 ± 0.08	1.54 ± 0.04	1.55 ± 0.02
Cholesterol (mmol/L)	2.2 ± 0.1	1.8 ± 0.1**	1.9 ± 0.1	2.0 ± 0.1**	1.6 ± 0.1	1.7 ± 0.1**	1.8 ± 0.1	1.8 ± 0.1
LDL cholesterol (mmol/L)	0.18 ± 0.02	0.16 ± 0.01***	0.11 ± 0.01***	0.12 ± 0.01***	0.11 ± 0.01*	0.11 ± 0.01**	0.12 ± 0.01*	0.13 ± 0.01**
HDL cholesterol (mmol/L)	1.35 ± 0.07	1.05 ± 0.03***	1.19 ± 0.08	1.13 ± 0.09	0.95 ± 0.05***	0.96 ± 0.09***	1.12 ± 0.07	1.10± 0.09
HDL/total cholesterol	0.61 ± 0.01	0.59 ± 0.02	0.62 ± 0.04	0.56 ± 0.02**	0.59 ± 0.01	0.56 ± 0.01**	0.59 ± 0.01	0.60 ± 0.01
Triacylglycerols (mmol/L)	1.43 ± 0.08	1.22 ± 0.09	1.17 ± 0.12	1.30 ± 0.18	1.07 ± 0.12**	1.29 ± 0.11	1.29 ± 0.11	1.29 ± 0.08
Glucose (mmol/L)	13.1 ± 0.9	9.0 ± 1.9*	12.3 ± 0.8	11.4 ± 0.8	12.0 ± 1.2	13.6 ± 1.0	13.5 ± 0.6	13.4 ± 1.0
Creatinine (μmol/L)	57 ± 3	52 ± 4	69 ± 3*	60 ± 3	52 ± 4	52 ± 5	55 ± 4	56 ± 4
Creatine kinase (U/L)	212 ± 37	777 ± 157***	166 ± 42	220 ± 42	402 ± 57	226 ± 38	356 ± 68*	365 ± 59*
ALT (U/L)	67 ± 7	93 ± 13*	79 ± 13	68 ± 8	65 ± 14	65 ± 10	62 ± 5	62 ± 3
AST (U/L)	145 ± 18	239 ± 37**	123 ± 14	122 ± 17	123 ± 26	136 ± 35	123 ± 16	117 ± 9

[a] Significant difference from the control group. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (one-way ANOVA).

Russula spp groups (3 and 9 g/kg/day) and at the same level in the *C. cibarius* groups (Table 1). The absolute or relative organ weights did not differ because of the exposures (Tables 1 and 2).

The plasma CK activities were higher in the *p*-phenylenediamine group and in the experimental groups receiving *Russula* spp at 3, 6, and 9 g/kg/day, *C. cibarius* at 3 and 9 g/kg/day, *A. ovinus* at 9 g/kg/day, and *L. versipelle* at 6 and 9 g/kg/day, compared with the control mice (Tables 1 and 2). The *p*-phenylenediamine exposure also caused reductions in the plasma glucose, total cholesterol, and HDL cholesterol concentrations. *A. ovinus* at 6 g/kg/day and *L. versipelle* at 3 g/kg/day also caused a reduction in the plasma total cholesterol concentrations compared with the control group. The plasma LDL cholesterol concentrations were lower in the *A. ovinus* and *L. versipelle* groups at all doses. The plasma triacylglycerol concentrations were lower in all the *Russula* spp groups and in the *A. ovinus* group at 9 g/kg/day. The plasma ALT and AST activity levels were higher in the *p*-phenylenediamine group, and the AST activity was higher in the *Russula* spp groups at 3 and 9 g/kg/day, and the *C. cibarius* at 9 g/kg/day group. In the histologic samples, no pathologic findings were observed in the liver or muscle tissues compared with the findings in the control mice.

Discussion

Historically, mushroom species that are considered edible have been consumed without harmful effects for centuries or millennia. Because of this, the specific chemical compounds causing toxic effects or milder irritant symptoms have been characterized mainly in species considered inedible or poisonous. Previously, Bedry *et al.* (1) observed elevated plasma CK activities in 12 French cases of delayed rhabdomyolysis requiring hospitalization after the consumption of at least three consecutive meals containing *T. flavovirens*, a species previously considered harmless. The same was observed in three Polish patients (2, 3). Furthermore, *T. flavovirens* extracts at 4–6 g/kg/day (1) or dried pulverized *T. flavovirens* or *B. edulis* at 9 g/kg/day for 5 days induced similar responses in experimental mice (4). Repeated meals or a relatively long period of consumption (5 d in this study) seem to be required for the toxic effects to occur (3, 4). The age of the individual could also be of importance (3). In addition, a previous study states that the storage of *T. flavovirens* extracts at –20°C for 12 months rendered the consumption of this species harmless in mice (11). The present study shows that the toxic effects are probably more widespread than previously assumed and that the same or a similar chemical compound responsible for myotoxicity seems to be present in many phylogenetically relatively distant mushroom genera. In addition, the chemical substance responsible for these responses does not seem to be very easily degraded, unlike suggested previously (11). In fact, after drying the mushrooms at +60°C and subsequent storage at room temperature for several months, they still elicited increased CK activities and, in some cases, increased plasma levels of indicators of liver or kidney damage.

The observed increases in the plasma CK activities caused by the ingestion of various mushroom species (up to 1334 U/liter) were approximately of the same magnitude as in previous studies conducted with *T. flavovirens* (1, 4). There was also a relatively high variation between the mice, especially in the 9 g/kg/day groups of the present study, indicating that the effect also depends on the individual organism and that some individuals are more susceptible than others are. The responses elicited by the different mushrooms differed in the dose required as well as in the CK values. The russulas seemed to be the most potent in this respect, because increased CK activities could be detected at 3 g/kg/day and the elevation in the CK activities was the most dramatic (212 ± 37 vs. 1334 ± 445 U/liter; or 6.3-fold higher than in the control mice). Increased CK activities could also be detected from *C. cibarius* consumption at 3 g/kg/day, whereas the responses to *A. ovinus* and *L. versipelle* were more modest and detected only at higher doses. The lack of a clear dose response in the *C. cibarius* groups is hard to explain. However, in all of the experimental groups, there were individual mice with no biochemical signs of tissue damage, i.e., increased plasma CK or transaminase activities. The high individual variability in the responses can thus partly explain the lack of a dose response, especially because the group size in this study was modest.

The absence of significant muscle fiber disorganization or, for instance, fatty liver degeneration in the histologic samples, indicates that the toxicity of these mushroom species is moderate even at these high levels of ingestion. However, the lack of visible histologic changes can also be a result of the relatively short duration of the exposure (5 d). It has been solidly established that increased CK activities caused by myotoxicity do not require gross myocyte damage visible in light microscopy (12). For instance, clearly increased CK activities in muscular dystrophies and alcoholic myopathy occur because of increased permeability of muscle cell membranes before any damage can be detected in histologic samples. It is possible that the same mechanism is responsible for the clinical findings of the mice in the present study.

In addition to elevated CK activities, the plasma AST activities were also higher after *Russula* spp ingestion at 3 and 9 g/kg/day and *C. cibarius* ingestion at 9 g/kg/day, with elevations comparable to those caused by *p*-phenylenediamine. In addition, the experimental animals of these groups had higher relative water intakes than the control mice, which was probably caused by the organism trying to dilute the toxins acquired *via* nutrition. These data must be taken as an indication of incipient liver damage caused by the consumption of these mushrooms. Because these two mushroom genera caused the strongest response on the plasma CK activities and the liver transaminases, it can be

hypothesized that russulas and *C. cibarius* would be more detrimental if consumed on a regular basis at a high daily dose. An additional sign of possible toxicity is the increased plasma creatinine concentration in the *C. cibarius* group at 6 g/kg/day and in the *A. ovinus* group at 3 g/kg/day. This can be a sign of incipient kidney dysfunction reminiscent of *R. subnigricans* poisoning (6), suggesting that the causative agent or agents are not only myotoxic (1, 4), but can cause also liver or kidney damage at higher doses. In the case of the russulas, the results of this study are supported by a previous report on the outbreak of rhabdomyolysis caused by *R. subnigricans*. In these human cases, additional symptoms included renal failure and circulatory shock accompanied by increased activities of ALT, AST, and CK (6). *R. subnigricans* has been found to contain several cytotoxic substances (7, 8). These include chlorinated phenyl ethers, called russuphelins A–F, of which russuphelins A, B, C, and D exhibit cytotoxic activity *in vitro*. In the future, the presence of these or similar molecules in the studied mushroom species should be investigated.

The mushroom species examined in this study are considered edible, tasty, or even very tasty species (13), with no previous reports of alleged toxicity. However, some slightly mutagenic effects in *Salmonella* tests have been noted in *C. cibarius* and *Boletus* sp (14, 15). To our knowledge, *A. ovinus* has not been tested for mutagenicity. In addition, the previous reports regarding the high mutagenicity of Russulales (14, 15) have been conducted mostly on *Lactarius* spp (milk caps), which emit a bitter-tasting latex, and are considered inedible in Western Europe and eaten in Eastern Europe only after thorough rinsing or boiling. In contrast, no mutagenic effects have been found in mild russulas. In this context, the finding of myotoxicity in these species may seem alarming. However, the equivalent amount of fresh mushroom to be consumed would be 1.30–4.49 kg/day for a 70-kg person for 5 consecutive days, which would require the ingestion of wild mushrooms at a rate rarely, if ever, encountered. However, if the human dose is corrected for body surface area differences using the body surface area constants of humans and mice (16), the human doses would be 106–365 g/day for a 70-kg person, in the range that can be realistically consumed.

In addition to alleged toxicity, wild mushrooms have also been recently examined for possible beneficial effects on the plasma lipid profile (17), showing reductions in the serum total and LDL cholesterol concentrations in laboratory rats (*Rattus norvegicus*) after the consumption of maitake (*Grifola frondosa*), shiitake (*Lentinus edodes*), and enokitake (*Flammulina velutipes*) fibers for 1–4 weeks. Similar findings were present in this study, because the plasma LDL cholesterol concentrations were lower than in the control mice in the *A. ovinus* and *L. versipelle* groups at all doses. In addition, due to *A. ovinus* exposure at 9g/kg/day, the plasma triacylglycerol concentrations were lower in all of the *Russula* spp groups. In fact, antioxidative compounds have been found to be present in *A. ovinus* (18). These effects would be considered beneficial from the point of view of human nutrition. Thus, the sum of these detrimental and beneficial effects is hard to define and no specific recommendations can be made based on the results of this study. However, it must also be emphasized that the equivalent amount of fresh mushrooms to be consumed by a 70-kg person would be 4.15 kg/day for *A. ovinus*, according to the results of this study.

The present study enforces the hypothesis that the previously detected myotoxic effect of *T. flavovirens* (1) is not specific to one mushroom species (4). Similar elevations in the plasma CK activities and additional effects on the liver transaminases and plasma creatinine were elicited by diverse mushroom genera used in this study. The dose required for these effects could be, in some cases (*Russula* spp), lower than 3 g/kg/day. Toxic effects could also be observed in the indicators of liver and kidney damage similar to previous studies on mushroom-triggered rhabdomyolysis (6). However, these findings should not be interpreted without caution. Despite these novel findings, the fact remains that the consumption of these wild mushrooms has been considered safe for millennia. In fact, the results also enforce previous findings that the harmful effects probably require prolonged daily exposure and high amounts of ingested mushroom to manifest. These results cannot be directly applied to the most popular cultivated mushrooms, *Agaricus bisporus* and *L. edodes*. These species should also be investigated because the present study indicates that the observed toxicity could be present in most, if not all, mushroom genera. A thorough screening of the most popular wild mushrooms should be carried out to investigate what the causative agent is, which are the genera and species with myotoxic substances, and what are the doses and durations of exposure required for toxic effects.

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1. Bedry R, Baudrimont I, Deffieux G, Creppy EE, Pomies JP, Ragnaud JM, Dupon M, Neau D, Gabinski C, De Witte S, Chapalain JC, Godeau P. Wild-mushroom intoxication as a cause of rhabdomyolysis. New Engl J Med 345:798–802, 2001.
2. Chodorowski Z, Waldman W, Sein Anand J. Acute poisoning with Tricholoma equestre. Przegl Lek 59:386–387, 2002.
3. Chodorowski Z, Sein Anand J, Grass M. Acute poisoning with Tricholoma equestre of five-year old child. Przegl Lek 60:309–310, 2003.
4. Nieminen P, Mustonen A-M, Kirsi M. Increased plasma creatine kinase activities triggered by edible wild mushrooms. Food Chem Toxicol 43: 133–138, 2005.
5. Parker H. Alaska's mushrooms, a practical guide. Portland: Alaska Northwest Books, 1994.
6. Lee PT, Wu ML, Tsai WJ, Ger J, Deng JF, Chung HM. Rhabdomyolysis: an unusual feature with mushroom poisoning. Am J Kidney Dis 38, E17:1–5, 2001.
7. Takahashi A, Agatsuma T, Matsuda M, Ohta T, Nunozawa T, Endo T, Nozoe S. Russuphelin A, a new cytotoxic substance from the

mushroom Russula subnigricans Hongo. Chem Pharm Bull 40:3185–3188, 1992.

8. Takahashi A, Agatsuma T, Ohta T, Nunozawa T, Endo T, Nozoe S. Russuphelins B, C, D, E and F, new cytotoxic substances from the mushroom Russula subnigricans Hongo. Chem Pharm Bull 41:1726–1729, 1993.
9. Korhonen M. Uusi sienikirja [The new book of mushrooms]. Helsinki: Otava, 1986.
10. Breitenbach J, Kränzlin F. Fungi of Switzerland. Boletes and agarics, 1st part. Lucerne: Edition Mykologia, Vol 3, 1991.
11. Chodorowski Z, Sznitowska M, Wisniewski M, Sein Anand J, Waldman W, Ronikier A. Tricholoma equestre—animal toxicity study [in Polish with English abstract]. Przegl Lek 61:351–352, 2004.
12. Zimmerman HJ, Henry JB. Serum enzyme determinations as an aid to diagnosis. In: Davidsohn I, Henry JB, Eds. Clinical Diagnosis by Laboratory Methods (15th ed.). Philadelphia: W.B. Saunders, pp837–869, 1974.
13. Gerhardt E. Der grosse BLV-Pilzführer für unterwegs. München: BLV, 1997.
14. Grüter A, Friederich U, Würgler FE. The mutagenicity of edible mushrooms in a histidine-independent bacterial test system. Food Chem Toxicol 29:159–165, 1991.
15. Sterner O, Bergman R, Kesler E, Magnusson G, Nilsson L, Wickberg B, Zimerson E, Zetterberg G. Mutagens in larger fungi. I. Forty-eight species screened for mutagenic activity in the Salmonella/microsome assay. Mutation Res 101:269–281, 1982.
16. Collins JM. Prospective predictions and validations in anticancer therapy. In: Pharmacokinetics in Risk Assessment: Drinking Water and Health. Washington, D.C.: National Academy Press, Vol 8:pp431–440, 1987.
17. Fukushima M, Ohashi T, Fujiwara Y, Sonoyama K, Nakano M. Cholesterol-lowering effects of maitake (Grifola frondosa) fiber, shiitake (Lentinus edodes) fiber, and enokitake (Flammulina velutipes) fiber in rats. Exp Biol Med 226:758–765, 2001.
18. Nukata M, Hishimoto T, Yamamoto I, Iwasaki N, Tanaka M, Asakawa Y. Neogrifolin derivatives possessing anti-oxidative activity from the mushroom Albatrellus ovinus. Phytochemistry 59:731–737, 2002.