



**SAFETY OF AMINO ACIDS USED
AS DIETARY SUPPLEMENTS**

July 1992

Prepared for

CENTER FOR FOOD SAFETY AND APPLIED NUTRITION
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
WASHINGTON, DC 20204

under

FDA Contract No. 223-88-2124
Task Order No. 8

FASEB

LIFE SCIENCES RESEARCH OFFICE
FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY

9650 Rockville Pike
Bethesda, MD 20814-3998

**SAFETY OF AMINO ACIDS USED
AS DIETARY SUPPLEMENTS**

July 1992

Prepared for
CENTER FOR FOOD SAFETY AND APPLIED NUTRITION
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
WASHINGTON, DC 20204

under
FDA Contract No. 223-88-2124
Task Order No. 8

edited by
S. A. Anderson R.D., Ph.D.
D. J. Raiten, Ph.D.

LIFE SCIENCES RESEARCH OFFICE
FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY
9650 ROCKVILLE PIKE
BETHESDA, MARYLAND 20814-3998

2a. Arginine

a. Background

Arginine is a glyco-genic amino acid metabolized via ornithine to α -ketoglutarate which can either be used for glucose synthesis or catabolized to produce energy in the TCA cycle (Rodwell, 1990a). Arginine is needed for tissue protein synthesis, biosynthesis of amino acids and polyamines, provision of the amidino group in creatine synthesis, and ammonia detoxification via the urea cycle (Rodwell, 1990b,c). The amino acid is also a secretagogue for several endocrine glands, stimulating the secretion of pituitary growth hormone and prolactin, pancreatic insulin, somatostatin, and polypeptide, and adrenal catecholamines. Arginine is a dispensable amino acid; however, in children with congenital defects of one of the urea cycle enzymes (other than arginase), arginine becomes an essential amino acid, and supplementation of the low-protein diets prescribed for these children is required indefinitely (Brusilow and Horwich, 1989).

L-Arginine is synthesized from a urea cycle intermediate, citrulline, primarily in the liver and to a lesser extent in the kidney. Many of the biologic and pharmacologic effects of L-arginine are shared with the urea cycle intermediates L-ornithine and L-citrulline. L-Arginine shares many transport mechanisms in the body with L-lysine. For example, together with ornithine and histidine, they share the system Y carrier for transport across cellular membranes (Skeie et al., 1990). Investigations in pigs have shown that arginine and lysine, as free amino acids, compete for absorption in the small intestine (Buraczewski et al., 1970). Similarly, studies in dogs have shown that arginine and lysine compete for reabsorption from the renal tubules (Kamin and Handler, 1951; Webber et al., 1961). Urinary excretion of lysine was also increased by excess dietary arginine in pigs (Southern and Baker, 1982).

L-Arginine, but not D-arginine, has been reported to lead to the generation of nitric oxide and citrulline via a deaminase-like enzyme system (Calver et al., 1990; Moncada et al., 1989). Nitric oxide may act as a neurotransmitter in brain. In abnormally high concentrations, it can be toxic to neurons (Bredt and Snyder, 1992). Investigations in vitro systems, animals, and humans indicate that arginine can serve as a nitrogen source for formation of nitric oxide in brain and other cells and in endothelium of blood vessels (Bredt and Snyder, 1992; Leaf et al., 1989; Palmer and Moncada, 1990). However, it remains to be determined whether manipulation of oral intakes of L-arginine can affect nitric oxide synthesis. It has been speculated that arginine might exert some effects on the immune system by increasing nitric oxide levels (Barbul et al., 1990).

Oral or intravenous administration of the hydrochloride salt of arginine may result in acidosis. Thus, in any situation in which arginine is administered as the hydrochloride salt, evaluation of responses or effects due to acidosis need to be distinguished from possible effects of the amino acid itself. Intraperitoneal injection of arginine hydrochloride has been reported to protect rats (duRuisseau et al., 1956; Greenstein et al., 1956; Gullino et al., 1956) and dogs (Najarian and Harper, 1956) against ammonia intoxication induced by administration of ammonium acetate or toxic doses of other amino acids. A neutralized solution of L-arginine given intraperitoneally at a dose of 6 mmol/kg prevented the rise in blood and brain ammonia concentrations in rats induced by insulin, pentylenetetrazol, or ammonium salts and protected the animals against convulsions (Roberge and Charbonneau, 1969). In premature infants, supplemental arginine (free base) at 0.5 to 2 mmol/kg body weight daily has been shown to counteract the hyperammonemia commonly seen in these children (Batshaw et al., 1984; Heird et al., 1972).

b. Animal Studies

Changes in food intake and body weight: Growth depression has resulted in rats fed low-protein diets containing 4, 5, and 7.5 percent of added arginine (Harper et al., 1966; Sauberlich, 1961;

Schimke, 1963). Growth depression associated with excess arginine was lessened when the protein content of the diet was increased and when the protein quality was improved (Harper et al., 1970; Muramatsu et al., 1971).

Muramatsu et al. (1971) demonstrated that weight gain over a 3-week feeding period was depressed by 16 percent in male weanling Donryu rats fed a 10 percent casein diet with a 5 percent L-arginine supplement although food intake was similar to that of control animals. Liver weight and protein, DNA, and RNA content were not significantly different from controls. Supplementation of a 25 percent casein diet with 2 percent L-arginine as L-arginine hydrochloride improved post-trauma growth but did not result in improved nitrogen retention in male ARS Sprague-Dawley rats traumatized by hind leg fracture compared with traumatized rats fed the 25 percent casein diet without an amino acid supplement (Pui and Fisher, 1979).

Addition of 0.94 to 1.63 percent L-arginine as L-arginine hydrochloride to diets containing 1.03 and 1.2 percent lysine did not affect weight gain or feed intake of young growing pigs over a 28-day period unless lysine was insufficient in the diet (Hagemeyer et al., 1983). However, addition of 4 percent L-arginine free base to diets of young pigs decreased weight gain by 31 percent and food intake by 22 percent over a 16-day feeding period (Edmonds et al., 1987a,b).

Biochemical studies: Feeding of 5 percent L-arginine in a 6 percent casein diet to male weanling Sprague-Dawley rats for 4 weeks resulted in a 5-fold increase in plasma arginine (Sauberlich, 1961). Hyperkalemia and hypermagnesemia in the presence of acidosis developed in bilaterally nephrectomized male Sprague-Dawley rats injected intraperitoneally with 400 mg arginine hydrochloride, but not in nephrectomized controls. The isomer was not specified in this study. The authors interpreted this finding as a demonstration of an arginine-induced flux of these ions from intracellular to extracellular compartments (Whang et al., 1988).

Behavioral studies: Male rats of the Wistar and inbred CDR strains were given a saline control solution or L-arginine hydrochloride orally at dose levels of 100, 500, or 1000 mg/kg body weight (about 12, 60, and 120 mg L-arginine for a 150-g rat) 1 hour before behavioral trials for 5 or 7 days. CDR rats (a strain with poor learning capacity) were trained to avoid footshock presented on one side (the side the rat was in) of a two-compartment shuttle-box. A buzzer preceded the footshock by 5 seconds signalling the animal to move from one side of the box to the other. Avoidance behavior was greater in CDR rats given the highest dose than in controls. A similar finding was reported in a passive avoidance task (in which not making a response was associated with avoiding a shock) after 7 days of treatment with the highest dose of L-arginine. In the Wistar rats, conditioned avoidance behavior was not affected although there was an increase in ambulation (Drago et al., 1984).

Endocrine studies: Intravenous infusion of L-arginine (1 mmol/kg body weight or 1.7 g for a 10-kg dog) over 15 minutes resulted in significant increases in plasma insulin and glucagon concentrations in 4 fasting dogs (Rocha et al., 1972). However, in fasting male Sprague-Dawley rats, intragastric administration of arginine (142 mg/kg body weight or 36 mg for a 250-g rat) or an equimolar amount of aspartic acid did not result in an increase in plasma insulin, glucagon, or growth hormone although administration of an equimolar amount of arginine aspartate (250 mg/kg body weight or 62 mg for a 250-g rat) produced a significant increase in the concentration of growth hormone (Franchimont et al., 1984).

Immunological studies: Solutions for intravenous hyperalimentation supplying about 290 or 540 mg/day of L-arginine were administered to adult male Sprague-Dawley rats for 7 days (Barbul et al., 1985). With the higher level of arginine, wound healing was accelerated and thymic function was improved (increased thymic weight, total number of thymic lymphocytes/gland, and mitogenic reactivity of thymic lymphocytes to phytohemagglutinin and concanavalin A) (Barbul et al., 1985). Ingestion of L-arginine hydrochloride as dietary supplements at levels of 0.5, 1, 2, and 3 percent for 6 days significantly increased thymic weight, thymic lymphocyte content, and the in vitro reactivity

of thymic lymphocytes in CBA/J mice (Barbul et al., 1980). Responses were similar at all levels of supplementation (Barbul et al., 1980). Also in the CBA/J mouse strain, arginine supplementation (1, 2, or 4 percent in drinking water providing 60, 120 or 240 mg/day assuming 6 ml consumed; isomer not specified) resulted in significantly increased thymus weight, spleen cell mitogenesis, and inducible natural killer cell activity with 1 and 2 percent but not 4 percent supplementation (Reynolds et al., 1990). However, in other studies (Ronnenberg et al., 1991), ingestion of L-arginine hydrochloride at levels of 3 percent of the diet did not increase thymus weights and had little effect on lymphocyte proliferation or IL-2 production in healthy young or aged rats.

Survival rates decreased with increasing level of arginine supplementation (0, 2, 4, or 6 percent arginine hydrochloride in a parenteral formulation containing 5 percent protein) in 8 female Hartley guinea pigs with established sepsis (Gonce et al., 1990). Previous work by this group indicated greater resting metabolic expenditure and lower mortality rates with intragastric tube feedings supplemented with 1 and 2 percent arginine but not 4 percent arginine in burn-traumatized female Hartley guinea pigs for 14 days (Saito et al., 1987). Ear thickness response to a dinitrofluorobenzene challenge on post burn day 12 showed the best response in the group given 2 percent arginine. The authors reported that the lack of improvement seen with 4 percent arginine might be considered an adverse effect and suggested that amino acid imbalance and disturbed protein metabolism might be important mechanisms for an adverse effect of excess arginine.

Gross pathology: Mizunuma et al. (1984) reported marked pathological changes in pancreas and adipose tissue of male Wistar rats given a single intraperitoneal injection of L-arginine (5 g/kg body weight or 0.75 g for a 150-g rat). Over the 24 to 72 hours after injection, pancreatic acinar cells were destroyed selectively; no changes were observed in the islets of Langerhans. The changes were described as similar to pancreatic changes observed after excess L-methionine. The authors concluded that a decrease in protein synthesis in acinar cells resulting from an amino acid imbalance was responsible for the pancreatic damage. Over the same time course, peripancreatic, epididymal, omental, and retroperitoneal adipose tissue became necrotic and infiltrated with polymorphonuclear leukocytes. Serum lipase activity was significantly increased 24 hours after L-arginine injection.

Teratology and developmental studies: Daily intraperitoneal injection of 15 mg/kg body weight of L-arginine hydrochloride to 18 pregnant rats (about 2.5 mg/day in a 200-g rat) on days 1 to 6 of gestation resulted in hindlimb malformations in 43 percent of the fetuses of the arginine-treated dams (Naidu, 1973). Information on effects in the control group was not reported. The report included little experimental detail and it may not be reasonable to attribute teratogenic effects to the small amount of arginine administered.

c. Human Studies

Biochemical studies: The arginine salt of glutamic acid (arginine glutamate) has been used to treat acute hepatic encephalopathy in doses of 50 to 100 g given intravenously (Tobe, 1961; Davey, 1964). In these studies, 1 or 2 infusions of arginine glutamate were given to each patient. No side effects were seen apart from blisters at the site of infusion in 2 of 40 patients and mild side effects were reported at infusion rates exceeding 25 g/hour (Davey, 1964). However, oral administration of 25 g arginine glutamate to 3 fasting patients (2 with chronic alcoholism and 1 with episodic encephalopathy) resulted in an increase in blood ammonia concentration of at least 10 percent within 2 hours in all patients. Because of the consistent rise in blood ammonia, the investigators concluded that oral and intravenous administration of arginine glutamate did not produce equivalent effects. They further advised against oral administration of arginine glutamate to patients with liver disease.

Intravenous loading of L-arginine hydrochloride (0.5 g/kg body weight/30 minute supplying 0.4 g arginine/kg body weight) 4 hours after feeding in 7 infants 2 to 6 weeks of age (about 2 g of arginine in a 5-kg infant) resulted in an immediate marked increase in plasma arginine concentration, with a peak of about 7000 $\mu\text{mol/L}$ (normal range 50 to 100 $\mu\text{mol/L}$). Ornithine concentrations also increased markedly but lagged behind the increase in arginine; plasma concentrations of other amino acids did not change significantly. Ammonia and urea concentrations and acid-base balance (pH and standard bicarbonate levels) did not change significantly with arginine infusion (Kraus et al., 1976). It should be noted that the peak plasma concentration in this study was about 5 times higher than plasma levels in patients with hyperargininemia (see p. 123) and remained above 1000 $\mu\text{mol/L}$ for 2 hours after infusion. Lack of increase in plasma ammonia and urea levels was interpreted by the authors as evidence that infants were capable of metabolizing this load of arginine without overt adverse effects.

Metabolic effects of arginine administration and protein restriction in five patients with liver cirrhosis were studied by Baertl and Gabuzda (1959). Patients were infused intravenously over 3-hour periods with 20 g L-arginine hydrochloride daily for 6 days. Infusion of L-arginine hydrochloride did not have a significant effect on serum sodium, chloride, or potassium concentrations; however, effects on blood urea nitrogen and nonprotein nitrogen concentrations varied among the patients. Urinary chloride excretion was greatly increased and nitrogen, ammonium, and potassium excretion were also increased. In one patient also given 20 g L-arginine hydrochloride daily for 6 days or 16.5 g L-arginine base daily for 6 days, metabolic changes were similar to those described for intravenous infusion. Baertl and Gabuzda (1959) reported that there was no clinical intolerance to L-arginine hydrochloride infused intravenously or to L-arginine free base given orally. Daily oral ingestion of 20 g of L-arginine hydrochloride resulted in diarrhea in the one patient studied. In the absence of a control group of patients or administration of a placebo compound, these results should be interpreted with caution.

Intravenous administration of arginine hydrochloride (0.5 g/kg body weight given over 30 minutes or 35 g for a 70-kg person) to 15 normal adults resulted in a rise in blood potassium (from 4 mEq/L initially to 4.9 mEq/L 90 minutes after infusion) and a fall in blood phosphorus (from 3.3 mg/L initially to 2 mg/L 60 minutes after infusion) (Massara et al., 1979). Administration of this dose of arginine to 14 insulin-dependent diabetic subjects resulted in a pronounced increase in blood potassium concentration to pathological levels (5.6 to 6.5 mEq/L in 9 subjects) and a smaller but significant decrease in blood phosphorus concentration (from 3.5 to about 3.3 mg/L). No significant changes were observed in blood pH, plasma osmolality, or plasma aldosterone (Massara et al., 1981). In patients with renal and hepatic insufficiency, infusion of arginine hydrochloride also resulted in life-threatening hyperkalemia (Bushinsky and Gennari, 1978; Hertz and Richardson, 1972). Isomers were not specified in these studies.

Oral ingestion of L-arginine hydrochloride (100 mg/kg body weight [83 mg arginine/kg body weight]) by 10 fasted healthy human subjects 20 to 49 years of age resulted in significantly increased concentrations of ornithine and arginine compared with 8 control subjects. Glucogenic precursors (proline and alanine) did not accumulate (Iwasaki et al., 1987). Serum insulin concentration was significantly increased 20 minutes after ingestion and serum glucose and free fatty acid levels were significantly decreased after 1 hour. In contrast, ingestion of L-ornithine hydrochloride (100 mg/kg body weight [79 mg ornithine/kg]) by 13 subjects resulted in significantly increased plasma concentrations of proline, alanine, ornithine and arginine and decreased concentrations of valine and urea; however, serum insulin, glucose, and free fatty acid levels were not significantly different from the control group 1 hour after administration. Serum levels of growth hormone and cortisol were reported unchanged with both treatments but the data were not presented (Iwasaki et al., 1987). The report did not include mention of side effects resulting from administration of a single bolus of L-arginine or L-ornithine.

Endocrine studies: Intravenous administration of 30 g arginine (0.5 g/kg in children) over a 30-minute period has been given routinely in tests of human insulin response or pituitary function (Alba-Roth et al., 1988; Barbul, 1986; Casanueva et al., 1984; Cordido et al., 1990; Fajans and Floyd, 1972; Ferrero et al., 1980; Page et al., 1988; Goodner and Porte, 1972). Intravenous injection of 30 g L-arginine in 18 fasting healthy adults produced the largest increase in plasma insulin concentration of any of 10 single amino acids given. No adverse effects were reported (Floyd et al., 1966). Differences in arginine-induced responses in insulin and glucagon concentrations were reported for young adult and elderly subjects by Ferrero et al. (1980). No observations of adverse effects were noted in the reports of these studies.

In 8 healthy male subjects without history of cardiovascular disease, consumption of a meal containing 20 percent soybean oil, 20 percent casein, and 60 percent corn syrup solids supplemented with 3 g of arginine and 2 g glycine resulted in a significant increase in plasma glucagon and a nonsignificant decrease in plasma insulin in comparison with consumption of an unsupplemented meal; postprandial plasma cholesterol and triglyceride concentrations were similar with both meals (Sanchez et al., 1988).

Arginine aspartate was administered orally at a level of 250 mg/kg body weight (17.5 g/day arginine aspartate for a 70-kg male; 9.9 g/day of arginine and 7.6 g/day of aspartate) daily for 7 days to 5 healthy male subjects (Bessett et al., 1982). The slow wave sleep-related growth hormone peak was about 60 percent higher after 7 days of arginine aspartate administration than in the control period. Individual increases ranged from 24 to 162 percent. Nocturnal mean plasma prolactin concentration was also higher after arginine aspartate infusion. The hormonal changes were not accompanied by any detectable alteration of sleep organization (Bessett et al., 1982).

Immune function studies: Park et al. (1992) reported that the rate of tumor protein synthesis more than doubled in breast cancer patients given oral doses of L-arginine free base (30 g/day in 4 divided doses for 3 days) compared with breast cancer patients not given the amino acid. In addition to the significant increase in protein synthesis, the investigators reported a marked stimulation in the expression of the activation antigen Ki67 in the tumors of the L-arginine-supplemented patients. No difference was found in the plasma insulin concentration, which was considered an index of arginine-induced endocrine stimulation. Most of the tumors in these patients were categorized histologically as invasive ductal cell carcinomas. Mild diarrhea, which subsided when the supplement was no longer taken, was reported as a side effect in 2 of the 10 women given the supplement. These results are in direct contrast to studies in animals in which administration of L-arginine suppressed tumor growth (e.g., Barbul, 1986; Levy et al., 1954; Milner and Stepanovich, 1979; Tachibana et al., 1985).

In a 7-day study of the effect of oral ingestion of 30 g/day arginine hydrochloride on lymphocyte immune response measured *in vitro* as peripheral blood lymphocyte mitogenic reactivity in 21 healthy human volunteers, no differences in liver function, BUN, creatine, or blood glucose were found (Barbul et al., 1981). Nausea and diarrhea were reported by 2 and 3 subjects, respectively. These side effects responded to lowering the dose ingested at any one time; the total daily dose was not decreased. There was no control group for the study and dietary intake was not controlled or monitored.

The effect of oral arginine supplementation on wound healing and lymphocyte immune responses was studied in 36 healthy, nonsmoking human volunteers (Barbul et al., 1990). For 2 weeks, volunteers were given 30 g/daily arginine hydrochloride (24.8 g arginine), 30 g/day arginine aspartate (17 g arginine), or a placebo. Diet was not controlled during the experimental period. In the subjects supplemented with 30 g/daily of arginine hydrochloride, mild hyperchloremic acidosis developed with significant increases in serum chloride and significant compensatory decreases in serum potassium, calcium, and bicarbonate. No increases in blood urea nitrogen or creatinine levels were found with either form of arginine supplement. Plasma amino acid analyses showed significant increases in arginine and ornithine concentrations, and a decrease in lysine

concentration with both arginine supplements. Concentrations of other amino acids were not altered by the arginine supplements. During the study, several subjects reported side effects such as bloating, mild anorexia, and diarrhea (Barbul et al., 1990). These were not severe enough to interfere with regular activities. Incidence of all occurrences was 1 of 12 subjects given placebo, 3 of 12 given arginine aspartate, and 6 of 12 given arginine hydrochloride. Subjects were instructed to space intake of supplements throughout the day but this was not rigorously controlled. The investigators attributed the occurrence of side effects to the large osmotic load.

In studies of mitogenic reactivity of peripheral lymphocytes of normal subjects fed 30 g of L-arginine hydrochloride daily for 1 to 2 weeks, no adverse effects on liver function were identified. Diarrhea and nausea that occurred at higher doses did not recur with lower doses (Barbul et al., 1981).

In postsurgical patients, supplementation of an enteral diet with 25 g of L-arginine daily for 7 days significantly improved the mean CD₄ phenotype (percent T-cells) and the mean T-lymphocyte response to concanavalin A compared with a glycine-supplemented group given the same diet (Daly et al., 1988).

Tiwary et al. (1973) reported an apparent anaphylactic reaction in a child ten years of age following infusion of 100 ml of a commercially available 5 percent arginine hydrochloride solution (approximately 5 g arginine given over 12 minutes). All signs and symptoms of the reaction had abated in 2 hours. No description of treatment for the reaction was given.

Functional assessments: Intravenous infusion of 60 g of L-arginine in 500 ml of infusate over 40 to 50 minutes resulted in an increase in excretion rate of albumin from a baseline level of 8.6 $\mu\text{g}/\text{minute}$ to 142 $\mu\text{g}/\text{minute}$ in 5 healthy men (Mogensen et al., 1975). Administration of graded doses of L-arginine (3, 6, 9, or 12 g) to 6 healthy subjects resulted in significant increases in albumin excretion at each dose level (5.8 $\mu\text{g}/\text{minute}$ baseline, 9.4 $\mu\text{g}/\text{minute}$ with 3 g, 13.2 $\mu\text{g}/\text{minute}$ with 6 g, 21.6 $\mu\text{g}/\text{minute}$ with 9 g, and 33.9 $\mu\text{g}/\text{minute}$ with 12 g arginine). Dose-related significant increases in light chain immunoglobulin and β -microglobulin were also seen with increasing doses of L-arginine (Mogensen et al., 1975).

A single intravenous injection of 6 g L-arginine in 5 nonfasting young male subjects caused an immediate inhibition of tubular protein reabsorption, resulting in significantly increased urinary excretion of albumin, light chains, and beta-2-microglobulin (Mogensen and Sølling, 1977). The authors reported that the single injection of this amount of arginine did not result in untoward effects. The response to arginine was lower than the response to an equimolar injection of L-lysine, the most active of the compounds tested. Injection of 1.5 g L-arginine also significantly increased β -microglobulin excretion but injection of equimolar amounts of other L-amino acids (leucine, valine, proline, histidine, methionine, aspartic acid, glycine, serine, phenylalanine, or tryptophan) did not increase excretion of this protein. Intravenous injection of 2.4 g L-ornithine, which shares many of the biological effects of L-arginine, resulted in a significant elevation in excretion of light chains and β -microglobulin but not albumin. Injection of an equimolar amount (3.2 g) of citrulline, a precursor of arginine in the urea cycle, had no inhibitory effect on tubular reabsorption of any of the proteins (Mogensen and Sølling, 1977).

GFR, RPF, and plasma glucagon concentrations were compared when 30 g arginine hydrochloride (24.9 g arginine) was given orally or the same amount of arginine hydrochloride or glucagon (10 ng/kg body weight/minute) was given intravenously to 6 normotensive subjects with no history of renal disease (Smoyer et al., 1991). The peak GFR was higher with oral administration of arginine than with intravenous arginine or glucagon, despite a lower peak glucagon concentration. Both oral arginine and intravenous glucagon, but not intravenous arginine, significantly increased RPF. Plasma concentrations of the gastrointestinal hormones (gastrin, neurotensin, and pancreatic polypeptide) did not differ with either route of arginine administration (Smoyer et al., 1991). These findings suggest that when arginine supplements are given, the route of administration may be

important with respect to changes in renal function; however, care must be exercised in extrapolating between effects observed with bolus intravenous infusions and oral intakes of similar or smaller amounts.

Intravenous injection of both isomers of arginine have been reported to induce hypotension in humans (Calver et al., 1990; Nakaki et al., 1990). Doses producing hypotensive effects were 640 μmol (111 mg) given in 4 minutes to 10 healthy normotensive subjects and 2.4 mmol (415 mg)/kg body weight (30 g for a 70-kg subject) given over 30 minutes to 5 normotensive and 5 hypertensive subjects, respectively. Suggested mechanisms have included vasodilation mediated by endothelium-derived relaxing factor (EDRF) (Nakaki et al., 1990), by nitric oxide formed endogenously from L-arginine (Hishikawa et al., 1991), by other unspecified arginine-mediated vasodilation mechanisms (Calver et al., 1990), and arginine-mediated histamine release (Paton, 1990).

Eighteen males aged 27 to 57 years ingested 1 g each of arginine and ornithine daily in 2 divided doses taken apart from meals 5 days per week for 5 weeks in a randomized placebo-controlled, double-blind study of the effects of these amino acids on body composition in combination with a weight training program (Elam, 1988). With the combined treatment body mass and body fat were reduced significantly more than with weight training and placebo. No information was given on dietary intakes of the subjects.

In another study with the same experimental protocol, 22 males with a mean age of 37 years consumed the same doses of L-arginine and L-ornithine while participating in a strength-training program (Elam et al., 1989). Subjects were asked to maintain their usual eating habits. Total strength and lean body mass were increased significantly and urinary hydroxyproline excretion was decreased significantly in the group taking the amino acid supplements. Neither study included mention of side effects of the L-arginine and L-ornithine supplements (Elam, 1988; Elam et al., 1989).

Investigations of orally administered arginine (0.5 to 4 g/daily) for as long as 12 weeks as a treatment for male infertility have not shown consistent effects of arginine on sperm count or motility (De Aloysio et al., 1982; Pryor et al., 1978; Schachter et al., 1973; Tanimura 1967). Weight increase, digestive troubles, and sleepiness were reported as reversible, dose-related side effects in subjects treated with 9 or 18 g arginine aspartate (5 or 10 g arginine) daily for 80 days (De Aloysio et al., 1982).

Solomons et al. (1971) reported that there were "no important side effects" attributable to oral administration of buffered arginine with treatment of L-arginine (mixed in a ratio of 0.9 g L-arginine free base to 15 g of L-arginine hydrochloride) for 14 patients with cystic fibrosis (1 to 19 years of age) for 10 days. The maximum daily dose was 25 g, given in an attempt to improve fat absorption.

Side effects of nausea in 2 patients with chronic liver disease and slight facial stiffness and paresthesia in 1 of these patients were reported with intravenous infusion of arginine glutamate (13.5 g L-arginine and 12 g L-glutamic acid) at a more rapid rate than 25 g/hour (Davey, 1964). No side effects occurred when the rate of infusion was reduced.

Inborn errors of metabolism: Argininemia is a metabolic disorder resulting from a deficiency of arginase activity. In addition to high plasma levels of arginine, the enzyme deficiency results in increased plasma and urine concentrations of orotic acid, and intermittently, mild hyperammonemia (Brusilow and Horwich, 1989; Yoshino et al., 1982). Clinical manifestations of the disorder include recurrent vomiting, seizures, spastic diplegia, psychomotor retardation, and delayed physical growth (Kang et al., 1983). Elevated plasma levels of both arginine (as high as 1500 $\mu\text{mol/L}$ or 261 mg/L, normal range 50 to 100 $\mu\text{mol/L}$ or 9 to 17 mg/L) and ammonia (3 to 4 times normal levels) may be responsible for these occurrences. CSF concentrations of arginine (normal range 10 to 30 $\mu\text{mol/L}$ or 1.7 to 5 mg/L), as well as ornithine, aspartate, threonine, glycine, and methionine, are also

The dibasic amino acids share common transport systems at the blood-brain barrier and renal tubules. CSF lysine concentrations were reported to be low in one patient before, but not during, treatment with a diet low in arginine (Brockstedt et al., 1990). However, lysine loads (250 mg/kg body weight; isomer not specified) given to normal subjects or patients with hyperargininemia did not result in decreased plasma arginine concentrations (Kang et al., 1983; Michels and Beaudet, 1978; Snyderman et al., 1977). Oral lysine treatment (250 mg/kg body weight/day) of one child with hyperargininemia for 6 months resulted in an increase in arginine and a decrease in ornithine in CSF (Kang et al., 1983).

Results of biochemical and hematological investigations were reported in 1 untreated patient with hyperargininemia (plasma and CSF arginine concentrations of 907 $\mu\text{mol/L}$ and 78 $\mu\text{mol/L}$, respectively) (Brockstedt et al., 1990). Blood chemistry, including electrolytes, creatinine, protein-electrophoresis, creatine kinase, alkaline phosphatase, LDH, glucose and ceruloplasmin was within normal limits. The patient exhibited a mild hyperammonemia and a low plasma concentration of urea. Hemoglobin concentration, hematocrit, erythrocyte sedimentation rate, red cell morphology, platelet count, and leukocyte differentiation were reported to be normal in this subject.

d. Summary and conclusions

Endpoints: In animal studies, addition of 4 to 7 percent of L-arginine to the diet has resulted in growth depression of rats and pigs. One study in rats and one study in humans have shown little behavioral effect of arginine given orally; however, these studies were very limited in scope. Administration of arginine resulted in elevated plasma potassium concentrations in animals and humans, reaching pathological levels when about 30 g was given to persons with diabetes mellitus or renal insufficiency. Arginine is known to be a secretagogue for several endocrine glands; intravenous infusion and oral administration of 30 g arginine as a single dose resulted in hypotensive effects, stimulation of insulin, glucagon, and growth hormone secretion, and increased glomerular filtration rate in humans.

The rate of tumor protein synthesis and expression of an activation antigen were significantly increased in women with breast cancer given 30 g L-arginine for 3 days. These results are in direct contrast to results of animal studies showing decreased tumor growth. In studies of immune function, administration of 25 and 30 g/day of arginine has not produced changes in liver function or plasma biochemical parameters in healthy humans, although a mild metabolic acidosis was reported following oral administration of arginine hydrochloride in humans. Administration of arginine to rats orally and intravenously has enhanced certain aspects of immune function at levels of dietary addition as high as 3 percent by weight, but not at 4 percent, suggesting that there may be an upper limit for augmentation of immune function by arginine.

Investigations in children with argininemia suggest that high plasma and cerebrospinal fluid concentrations of arginine early in life result in adverse effects on neurological development and growth. It appears that effects of high levels of arginine may result from competition of arginine with lysine for uptake and utilization in tissues as well as by other mechanisms.

Safe levels of human intake: The safety of excess arginine may be affected by lysine intake as well as the total amount of protein consumed. Daily intakes of arginine and lysine from dietary protein are about 5.4 and 5.0 g, respectively, for a person consuming 100 g protein.

Arginine glutamate given intravenously in doses of 50 to 100 g had few side effects in patients with hepatic encephalopathy; however, oral doses of 25 g arginine glutamate resulted in increased blood ammonia concentration in patients with liver disease. Long-term supplementation of the diets of children with certain inherited disorders of the urea cycle with 0.4 to 0.7 g/kg daily of arginine free base has not resulted in adverse effects. Side effects were not reported with daily doses of 1 g

L-arginine in combination with 1 g L-ornithine given 5 days per week for 5 weeks. Intravenous infusion of 30 g of L-arginine hydrochloride in a single bolus has been used clinically to evaluate insulin and pituitary hormone secretion. Although intravenous administration appears to be well-tolerated, increased plasma concentrations of potassium, particularly in diabetic individuals and persons with renal insufficiency, is of concern.

Oral intake of arginine aspartate (5 and 10 g/day of arginine) over an 80-day period has been reported to result in dose-related weight increase, digestive troubles, and sleepiness. Oral intakes of about 20 to 30 g/day of L-arginine hydrochloride for 7 to 14 days have resulted in gastrointestinal side effects (nausea, bloating, mild anorexia, and diarrhea). However, results of a recent study indicated that ingestion of 30 g/day of L-arginine as the free base resulted in stimulation of tumors in women with breast cancer. Although most studies have suggested that ingestion of as much as 30 g/day of arginine produced few side effects, the report that tumor growth increased with daily ingestion of 30 g L-arginine raises concern about its use as a dietary supplement. The Expert Panel considered these results of particular importance because it is likely that a study of this type will not be repeated because of ethical considerations. Therefore, it is not possible to conclude that use of L-arginine as a dietary supplement is not associated with adverse health effects. The Expert Panel is well aware that L-arginine has numerous uses medically, including treatment of inborn errors of metabolism and other disorders. Such treatments under medical supervision are outside the scope of this report. L-Arginine as a dietary supplement should be used only under responsible medical supervision.