THE EFFECTS OF ASPARTAME AND EXERCISE ON TISSUE LIPID LEVELS AND BODY COMPOSITION OF GROWING MALE RATS

by

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INTRODUCTION

Consumer demand for dietary foods and beverages has risen sharply in recent years as a direct result of rapidly increasing numbers of calorie conscious individuals. More than 68 million Americans, age 18 and over, are currently using low-calorie foods and beverages; of this total, over 50 million consumers use aspartame-sweetened products regularly (1).

In considering the total population of weight-conscious Americans, an increasing number of this total are utilizing exercise as a method of weight control. It is reasonable to assume that many of these "exercisers" also incorporate or will incorporate the use of the low-calorie sweetener aspartame (4 kcal/g) in their diets as an added measure of weight control. It is therefore important to determine the physiological interaction between aspartame and exercise.

The purpose of this study was to determine if aspartame supplemented rats responded to exercise differently than those not fed aspartame. This objective was accomplished by comparisons within a 2 x 2 factorial design (n=9) where the main treatment effects were treadmill exercise and aspartame. Feed intake, weight gain, body composition, percent body fat and serum lipid levels were measured. It is hoped that information from the study will lead to a better understanding of the physiological consequences of combining exercise and aspartame use as a method for weight control.

REVIEW OF LITERATURE

I. Use of Aspartame in Foods

Chemical Composition of Aspartame. Aspartame (N-L-α-aspartyl-L-phenylalanine methyl ester) is a dipeptide ester containing the amino acids aspartic acid and phenylalanine. A methyl group is esterified to the carboxyl group of the phenylalanine (Fig. 1). The chemical nature of aspartame, being similar to that of normal dietary protein, allows it to enter normal metabolic pathways where it is oxidized for energy via oxidative metabolism and the tricarboxylic acid cycle or broken down for utilization in other biosynthetic reactions (Fig. 2) (2).

Physical Properties and Sweetness. Aspartame is an odorless, white, water-soluble powder. Solubility increases in acid and/or warm solutions. It has no bitter aftertaste and is approximately 180 to 200 times sweeter than an equal weight of sucrose. Aspartame in powder (dry) form is stable at temperatures up to 40 C. (104 F) for over a year. In solution, however, gradual decomposition occurs, resulting in breakdown to its diketopiperazine form and a subsequent reduction in sweetness. Decomposition is slow in acid solutions and more rapid in alkaline ones. The maximum stability of aspartame in solution is at pH 3.9 to 4.3. Twenty percent of its sweetness is lost in four and a half months at a pH of 4 (that of some soft drinks) at 20 C (68 F). In neutral solutions half the sweetness can be lost in hours.

Utility as a Nutritive Sweetener. Aspartame (commercially known as NutraSweet) is currently being utilized as a table top sweetener with the brand name of Equal as well as a sugar substitute in soft drinks, dry beverage mixes, presweetened cereals, gelatins, puddings and fillings, chewing gum, and flavored coffees. Its role as a sweetener is limited to

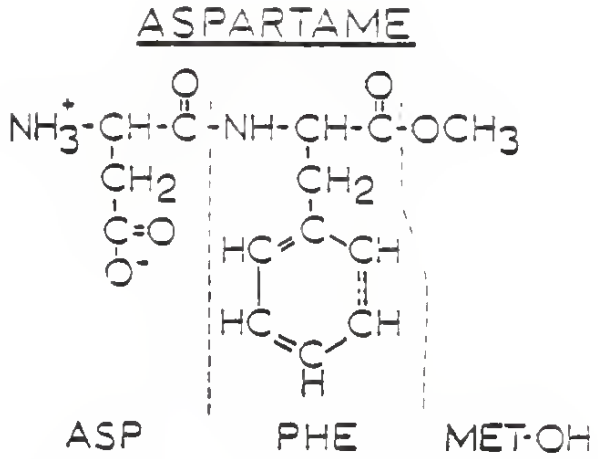


Fig. 1 Chemical composition of aspartame.

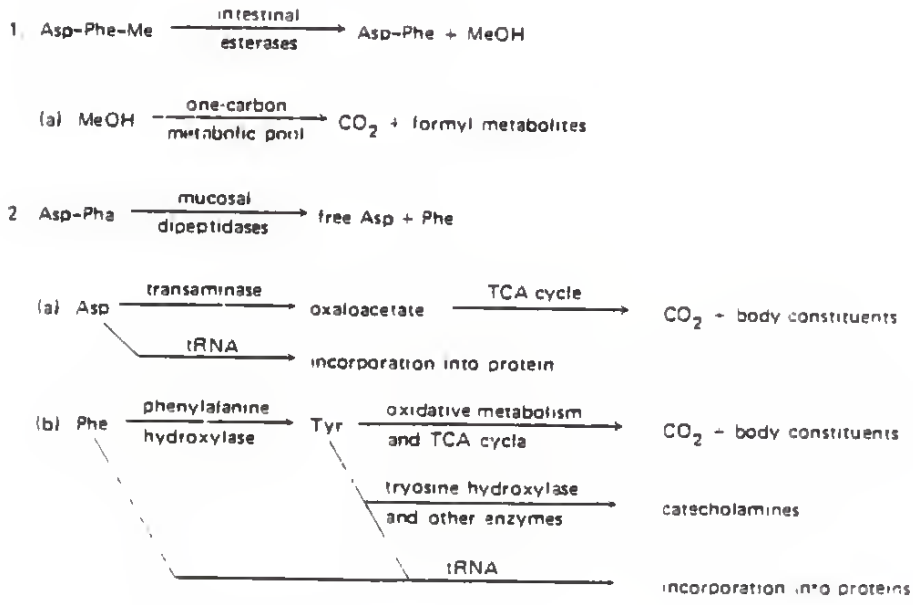


Fig. 2 Metabolism of aspartame

foods that do not require extensive cooking because sweetness decreases as aspartame hydrolyzes at high temperatures. Use in baked goods such as cakes, cookies and bread is restricted by its lack of bulk and structure. Also, it is not useful for sweetening nonacid liquids that are stored for long periods of time.

Current Consumption. With the rapid increase in numbers of calorie conscious Americans in recent years, there has been a concurrent growth in consumer demand for dietary foods and beverages. Over 50 million consumers use aspartame-sweetened products regularly (1). This consumption is not exclusively by consumers desiring to replace other sugar substitutes in the diet. Approximately 60% of Equal users did not previously use low-calorie tabletop sweeteners (3). This statistic suggests that sugar users are now some of Equal's best customers.

If all sucrose in the diet was replaced by aspartame, average consumption by a 60 kg (132 lb) person would be approximately 8.3 mg/kg body weight of aspartame per day, according to FDA estimates (4). Estimates of potential aspartame exposure were also derived from a study by the Market Research Corporation of America (MRCA) (5). In that study, food consumption data were gathered by MRCA from 12,337 individuals in 4000 households. Consumption of food products (both in-home and away-from-home) was recorded over randomly-spaced two-week periods for one year. The data thus presented an accurate estimate of what Americans actually eat. To project aspartame consumption, it was assumed that aspartame replaced sugar and other sweeteners in 30 product categories representing reasonable commercial uses of aspartame (including carbonated beverages). An intake of 34 mg/kg body weight was determined to represent the 99th percentile of potential theoretical daily usage exposure for all age groups combined.

II. Metabolism of Aspartame in Humans and Animals

Digestion and Absorption. Unlike non-nutritive sweeteners such as saccharin, aspartame is a nutritive sweetener and therefore enters normal metabolic pathways. Its components are either oxidized for energy or utilized in other biosynthetic reactions. Hydrolysis by esterases of the intestinal lumen yields the phenylalanine-aspartic acid dipeptide and methanol. The dipeptide is then converted by mucosal dipeptidases into its free amino acids. The methanol, phenylalanine and aspartic acid breakdown products are absorbed and metabolized by entering their usual metabolic pathways.

In a study on the metabolism of aspartame in monkeys, Ranney et al. (6) compared the metabolism of (^{14}C) aspartame labeled separately in the methyl, aspartyl and phenylalanine moieties to the metabolism of C-labeled methanol, aspartic acid, and phenylalanine. Each aspartame moiety was metabolized the same as its free counterpart, leading to the conclusion that aspartame was digested to its three components which were then absorbed as normal dietary constituents. A specific decription of the metabolism of each aspartame component follows.

Aspartic Acid Metabolism. Aspartic acid, one of the dicarboxylic amino acids, plays an important role in nitrogen and energy metabolism in the mitochondria. As illustrated in Figure 3, aspartate joins glutamate, the other dicarboxylic amino acid, in representing a major entry point to the tricarboxylic acid cycle (6).

The major pathway of free aspartic acid is its transamination with other α-ketoacids to produce oxaloacetic acid, an intermediate of the Krebs Cycle.

$$\text{Asp} + \alpha\text{-KG} \underset{\longleftarrow}{\xrightarrow{\text{aspartate transaminase}}} \text{OAA} + \text{L-glutamate}$$

$$\text{OAA} \rightarrow \text{KREBS CYCLE}$$

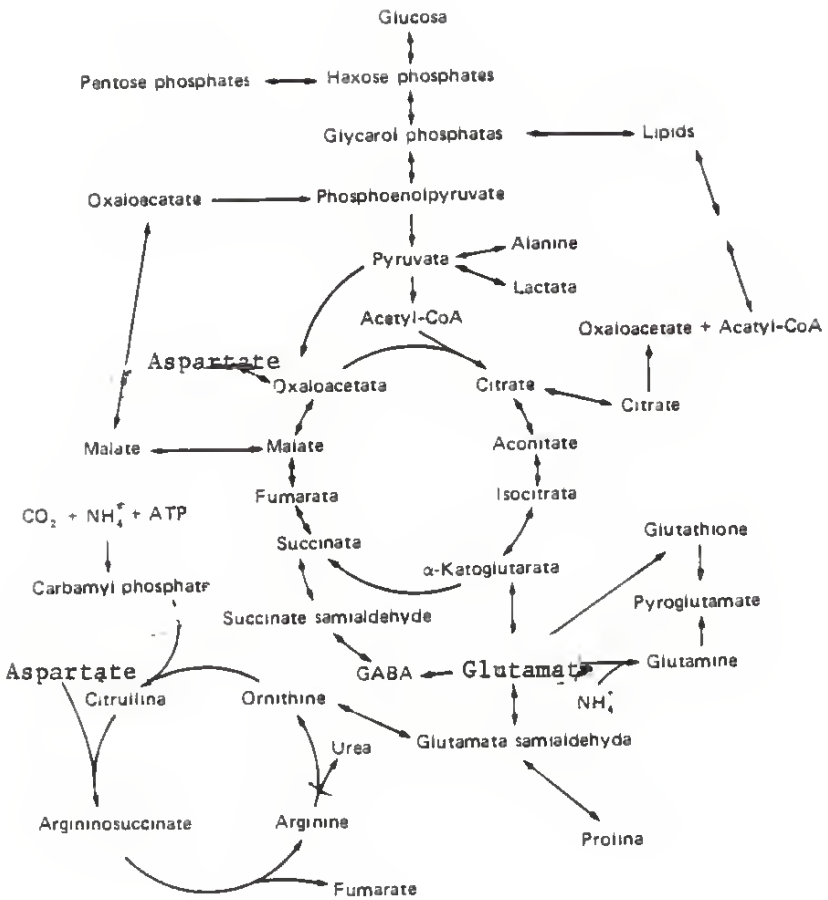


Fig. 3 Role of aspartic acid in nitrogen and energy metabolism. Ranney et al. (6).

Ranney et al. (6) showed that the resulting $^{14}CO_2$ excretion following (Asp-^{14}C) aspartame or (^{14}C) aspartic acid administration was similar in both rates and amounts of excretion. This suggests that the ingested aspartame rapidly produced free aspartic acid. Also, equal amounts of ^{14}C from each aspartic acid source (dietary and aspartame) were incorporated into plasma proteins. The percentage of aspartic acid not excreted as CO_2 ($\cong$30%) was converted to body constituents via the one-carbon metabolic pool.

Aspartate is also a vital substrate in the urea cycle, as the nitrogen atoms in urea originate from ammonium ions and aspartate (6) (Fig. 4). The ammonium ions are converted to carbamyl phosphate, which in turn condenses with ornithine to form citrulline. Aspartate condenses with the citrulline produced to yield argionsuccinate, which leads to the formation of arginine. The nitrogen originally present in aspartate is contained in the arginine, while the carbon skeleton remaining from aspartate is contained in fumarate. The nitrogen is finally transferred to yield urea when arginine is cleaved by arginase. Fumarate is converted to malate and oxaloacetate (OAA), which, upon transamination, yields aspartate, thus enabling repetition of the entire cycle. Because of the importance of aspartate to both the Krebs cycle and the urea cycle, it is a major amino acid in the mitochondria. Along with glutamate, it comprises approximately 50-70% of the total free amino acids found in the mitochondria (7).

Absorption of aspartate in the intestinal lumen depends upon its form when ingested. When ingested as a free amino acid in the diet, it is absorbed from the lumen by active transport. When ingested in protein or peptide form, the peptide enters the mucosal cells where it is hydrolyzed by specific intracellular hydrolases. Since aspartic acid in the form of aspartame is ingested as a dipeptide, absorption of aspartate as a protein or peptide will be the focus of this discussion.

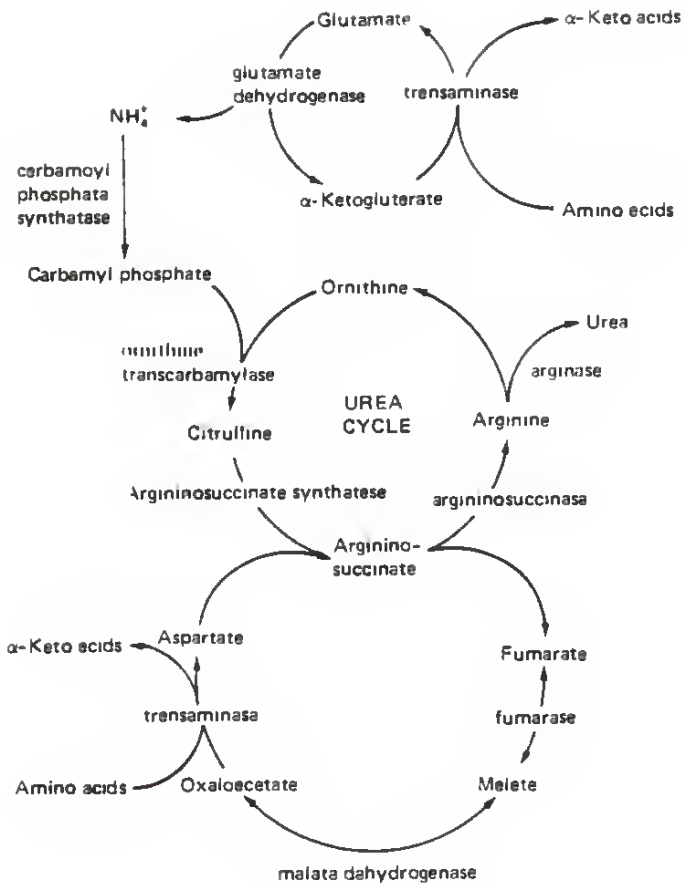


Fig. 4 Role of aspartic acid in the urea cycle. Ranney et al. (6).

Studies performed thus far suggest that peptide absorption may be the major route of protein absorption and that intestinal transport of amino acids from many dipeptides and tripeptides proceeds at a more rapid rate than from the equivalent amino acid mixtures (8, 9, 10, 11). That transport, in several mammals, including man, involves peptides composed of neutral, basic, and acidic amino acids. Matthews et al. (12) demonstrated that when a peptide is taken up, competition for transport between the amino acid components may be partially or completely avoided.

Nixon and Mauer (13), found that only the neutral amino acids and the dibasic amino acids (arginine and lysine) are quantitatively absorbed as amino acids. The imino acids (proline and hydroxyproline), glycine, and the dicarboxylic amino acids (glutamate and aspartate), all enter the intestinal mucosa as components of small peptides, where hydrolysis by specific intracellular peptidases can occur (14).

Gray and Cooper (15) reviewed the major mechanisms by which small peptides and amino acids are accommodated by the intestinal cell. Aspartate, present as a dicarboxylic peptide, was shown to be absorbed intact across the brush border and then hydrolyzed to its constituent amino acids by acidic peptidases once inside the mucosal cell. The resulting glutamate and aspartate free amino acids were then released to the portal circulation.

Although the majority of glutamate and aspartate peptides in protein are absorbed intact, specific transport sites for free glutamate and aspartate also exist in the gut, with maximal transport observed in the terminal ileum (16). Whether originating from protein hydrolysis or from ingestion as a free amino acid, aspartate is absorbed from the intestinal lumen by active transport which is at least partially dependent on sodium ions (17).

Phenylalanine Metabolism. The utilization and fate of dietary phenylalanine is illustrated in Fig. 5. Some phenylalanine present in the diet passes into the mucosal cell in the free form, leaving the remainder to be absorbed by the mucosal cell as small peptides, and then broken down into free amino acids (18).

Aspartame is a dicarboxylic peptide due to the presence of aspartic acid in its structure. As Gray and Cooper demonstrated (19), dicarboxylic peptides are absorbed intact across the brush border and then hydrolyzed to the constituent amino acids by acidic peptidases once inside the mucosal cell. Thus, phenylalanine in the form of aspartame would be absorbed as a dicarboxylic peptide rather than as a free amino acid.

After aspartame is hydrolyzed and phenylalanine is available in its free form, the absorbed phenylalanine passes to the liver and is utilized for protein synthesis in all tissues to which it is distributed by the systemic blood. Degradation in the liver occurs via hydroxylation of phenylalanine to tyrosine by phenylalanine-4-hydroxylase, which requires a pterin cofactor for completion (20). The tyrosine formed yields hydroxyphenyl-pyruvate, resulting in the formation of homogentisic acid and eventually, the production of CO_2.

$$\text{Phenylalanine} + O_2 + \text{tetrahydropterin} \xrightarrow{\text{hydroxylase}} \text{hydroxyphenylalanine (tyrosine)} + H_2O + \text{dihydroxypterin}$$

$$\text{Dihydroxypterin} + \text{NADPH} + H^+ \xrightarrow[\text{reductase}]{} \text{tetrahydropterin} + \text{NADP}$$

$$\text{Tyrosine} + \alpha\text{-KG} \xrightarrow{\text{amino transferase}} \text{hydroxyphenolpyruvate} + \text{glutamic acid}$$

A minor pathway of phenylalanine catabolism in normal subjects is represented by phenylalanine transamination to phenylpyruvate. Phenyl-

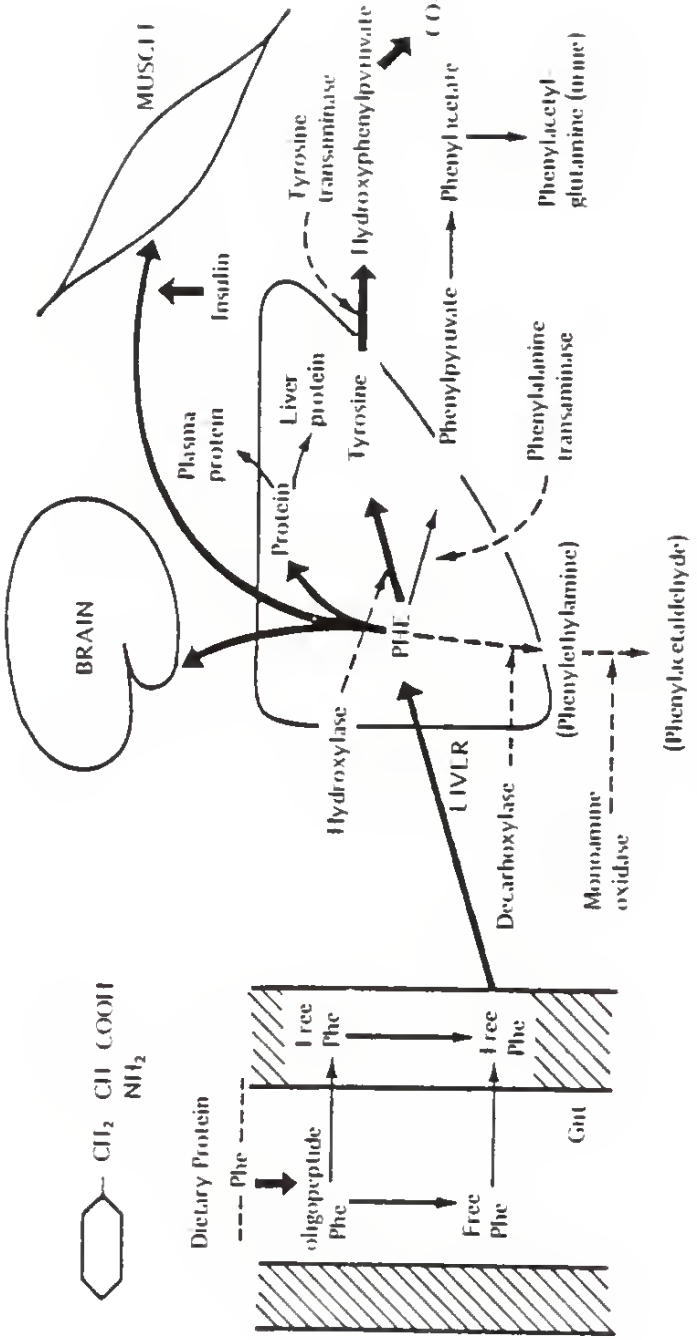


Fig. 5 Metabolism of phenylalanine. (18)

acetate, a product of phenylpyruvate, is not further metabolized in mammals and is excreted in small amounts in the urine (21, 22). This is the main pathway for phenylalanine degradation in phenylketonurics, who lack phenylalanine hydroxylase.

Ranney et al. (6) also showed that $^{14}CO_2$ excretion was the same for animals treated with (^{14}C) aspartame as for those given (^{14}C) phenylalanine. Disappearance of radioactivity of Phe-^{14}C aspartame and ^{14}C phenylalanine in plasma after 48 hours was slow. This suggested that the phenylalanine was incorporated into a metabolic pool with a slow turnover time, resulting in the incorporation of phenylalanine into plasma proteins and other normal body components. Approximately 20% of the phenylalanine was excreted as CO_2, urine or feces, thus leaving the remaining 80% to be incorporated into other body constituents. Because the major fraction of the phenylalanine was being retained by the body, Ranney et al. performed a second study (23) to determine if continued aspartame ingestion affected phenylalanine metabolism. After doses of 15 or 60 mg/kg were administered for ten days, it was found that disappearance of intravenously administered ^{14}C phenylalanine from the plasma was unaffected. The formation of tyrosine from labeled phenylalanine also was not affected. There was no effect on the rate of CO_2 conversion and incorporation into protein as well, which led to the conclusion that those doses of aspartame had not altered phenylalanine metabolism. The doses selected were chosen with extrapolation to humans in mind.

Methanol Metabolism. Absorption and metabolism of methanol from a methyl ester such as aspartame may differ from that of free methanol. Oppermann et al. (24) and Ranney et al. (25) compared the metabolism of methyl-labeled aspartame and radioactively labeled methanol in rats and

monkeys. Equivalent conversion of labeled methyl groups to CO_2 was observed with both compounds. However, less $^{14}CO_2$ was expired during the first hour after aspartame administration than after methanol treatment. The decreased $^{14}CO_2$ expiration in the aspartame group may be explained by the more rapid absorption of methanol from the stomach, the absorption of aspartame-derived methanol being slowed due to the necessary passage of aspartame into the small intestine before hydrolysis can occur to yield methanol.

$$\text{Asp - Phe - Me} \xrightarrow{\text{intestinal esterases}} \text{Asp - Phe} + \text{MeOH}$$

$$\text{MeOH} \xrightarrow[\text{metabolic pool}]{\text{one carbon}} CO_2 + \text{formyl metabolites}$$

Effect of Aspartame on Plasma Amino Acid Levels. In general, aspartame loading has been shown to have negligible effects on plasma amino acid levels. Stegink et al. (26) measured plasma amino acid levels of both normal and heterozygous phenylketonuric subjects after aspartame was administered (34 mg/kg) in orange juice. In both groups of subjects, plasma phenylalanine values (mean $\pm$ SD) increased from fasting levels (5.66 $\pm$ 1.21 μmoles/100 ml) to levels normally observed in the postprandial range (11.11 $\pm$ 2.49 μmoles/100 ml), returning to baseline within 8 hours. In heterozygous subjects, mean peak levels of phenylalanine were higher (16.03 $\pm$ 2.25 μmoles/100 ml). Maximum plasma levels, however, were not significantly above values noted postprandially, which indicated that aspartame-derived phenylalanine metabolism is slightly slower, yet adequately achieved, by the phenylketonuric heterozygote.

A significant increase in portal plasma glutamate and aspartate levels is possible by ingestion of large quantities of those amino acids (27). The increase in portal levels is proportional to the quant-

ity ingested, although some of the ingested dicarboxylic amino acid nitrogen will be converted to alanine.

Elevations in portal plasma glutamate and aspartate levels do not necessarily lead to an increase in peripheral plasma levels. The amino acid composition released to the peripheral circulation is controlled by the liver because the portal vein first carries blood to the liver. Stegink et al. (28) observed that the elevation of portal plasma glutamate and aspartate levels caused a significant uptake of those amino acids by the liver, a large portion of their carbon skeletons being released to the peripheral circulation as glucose and lactate.

Stegink et al. (29) also demonstrated the liver's ability to regulate plasma amino acid levels by measuring plasma amino acid levels in the neonatal pig after glutamate loading in water. At loads of 10 mg/kg body weight, no increase in peripheral plasma glutamate levels was evident while slight elevation occurred at a load of 100 mg/kg body weight. The only major increases were observed when the load was increased to 1 g/kg body weight.

Metabolism of dicarboxylic amino acids may differ in various species. Boaz et al. (30) indicated that in the neonatal rodent a different pattern of metabolites is released to the peripheral circulation after glutamate or aspartate loading in comparison to that in the primate. In the primate, the major metabolites released were glucose and lactate, while in the rodent, additional compounds were observed (α-KG when glutamate was administered, and OAA when aspartate was administered). This may be important when considering the susceptibility of the rodent to dicarboxylic amino acid-induced neuronal necrosis, in contrast to the primate, which is unaffected.

Plasma levels of amino acids after administration of aspartame (34 mg/kg body weight) in both normal and PKU heterozygous subjects were studied by Stegink et al. (31). No significant changes in plasma aspartate, asparagine, or glutamine levels were observed in either group, whereas a small rise in glutamate levels was noted in all subjects given aspartame. However, those levels remained within normal fasting levels and the increase in plasma glutamate was statistically insignificant.

Plasma phenylalanine levels rose significantly after aspartame loading in both groups of subjects (31), although levels were considerably higher in the PKU heterozygotes due to the decreased level of phenylalanine hydroxylase in the PKU liver. Maximal phenylalanine levels for normal subjects fell in the normal postprandial range for orally-fed human subjects. Maximal levels for PKU heterozygotes were slightly higher than normal postprandial values.

A small yet significant increase in plasma tyrosine levels occurred after aspartame loading in normal subjects at 30 minutes, with no significant rise observed in the plasma of heterozygotes (31). Levels were significantly higher in normal subjects than in PKU heterozygotes thirty to forty-five minutes after loading. Those differences were insignificant at 60 minutes.

Plasma levels of alanine and proline increased significantly in both groups (31). A response similar to this was observed in subjects receiving lactose in orange juice (32). Therefore, the increased levels of alanine and proline could not have been due to aspartame or aspartate, but most likely resulted from the orange juice or the stress of blood sampling (33).

Plasma levels of valine, isoleucine, and leucine decreased significantly 90 to 120 minutes after aspartame loading in both groups (31). This response was probably due to the carbohydrate content of the orange juice administered (34). Plasma levels of all other amino acids remained virtually unchanged from baseline levels after aspartame loading (31).

Toxicity. Questions concerning the safety of aspartame have arisen due to the possibility of toxic effects of the constituent amino acids, aspartate and phenylalanine (35, 36). These amino acids, like all chemical substances, may be toxic at very high dosages. From studies done thus far, however, it is evident that toxicity occurs only under conditions where blood levels of aspartate and phenylalanine are grossly elevated (in normal subjects).

Stegink et al. (26) observed effects of aspartame loading upon plasma amino acid levels in PKU heterozygotes and normal subjects. Plasma aspartate levels were unchanged after administration of 34 mg/kg aspartame in normal subjects and PKU heterozygotes, which indicated rapid metabolism of the aspartate present in aspartame. It is therefore unlikely that the aspartate content of aspartame administered at that dose poses any threat of toxicity to either normal persons or PKU heterozygotes.

Plasma phenylalanine levels did increase after aspartame loading in both normal and PKU subjects. Maximal phenylalanine levels for normal subjects however, were in the normal postprandial range for orally-fed human subjects. Maximal phenylalanine levels in PKU heterozygotes (16.03 ± 2.25 μmoles/100 ml) were slightly above the normal range. The developing homozygous infant appears to tolerate continued exposure to

levels higher than this range, so it appears that little risk, if any, is involved (37). That conclusion seems especially reasonable when the load of aspartame which was administered is considered. The 34 mg/kg dose of aspartame administered was estimated to represent the 99.9th percentile of ingestion when aspartame sweetness replaced all dietary sucrose sweetness (38). That margin of safety should prevent toxicity from aspartame consumption, even in PKU heterozygotes.

Administration of large doses of glutamate and aspartate has produced neuronal necrosis in infant mice (39, 40). Plasma glutamate and aspartate must be enormously elevated, however, before any lesions are observed. Plasma levels must reach 60 to 70 µmoles/100 ml before the first sign of neuronal necrosis begins. Therefore, even the acutely sensitive mouse is able to withstand plasma levels of up to five times normal aspartate and glutamate levels.

In comparison, plasma glutamate plus aspartate levels up to 500 µmoles/100 ml did not cause neuronal necrosis in the infant primate (41). Neuronal damage or toxicity resulting from dicarboxylic amino acid ingestion therefore requires two factors: 1) a sensitive animal species (rodent) and 2) markedly elevated plasma glutamate and aspartate levels. The aspartame dose studied did not significantly increase plasma glutamate and aspartate levels, and thus, this dose (34 mg/kg body weight) should present no risk of toxicity to man.

Uptake by Body Organs.

Liver. The liver is the primary site of utilization for both aspartic acid and phenylalanine (table 1). The availability of amino acids to the liver is increased by raising portal venous concentrations of amino acids, or by accelerating amino acid transport into hepatic

Table 1. Classification of the amino acids

Class	Transport system and affinity[a]		Primary site[b] of utilization
	L	A	
Basic			
Lysine		Basic	L
Arginine			L
Neutral			
Leucine	4	1	M,B
Isoleucine	4	1	M,B
Valine	4	1	M,B
Phenylalanine	4	1	L
Tyrosine	2	2	L
Methionine	4	4	L
Tryptophan	2	2	L
Histidine	2	2	L
Threonine	2	3	L
Alanine	1	3	L
Serine	1	3	L
Cystein	-	-	L
Proline	0	3	L
Glycine	+/-	2	L,K
Asparagine	-	-	L
Glutamine	2	2	G,K
Acidic			
Aspartic acid		Acidic	L
Glutamic acid			L,M

[a]The amino acids are generally transported by four separate carriers: the basic, acidic, and two neutral transport systems, the leucine-preferring (L) and the alanine-preferring (A) systems. The relative affinity (from 0 to +4) of the L and A systems for the respective amino acids is listed according to Christensen (45).

[b]The primary sites of amino acid metabolism are based on net uptake (arteriovenous differences) data in the 16- to 24-hr fasted organism (46, 47, 48, 49). L, liver. G, gut. M, muscle. K, kidney. B, brain.

cells. Fasting, for example, has a stimulatory effect on hepatic amino acid transport (42). Thus, fasting would enhance liver uptake of aspartic acid and phenylalanine. The insulin antagonists, epinephrine, glucagon and growth hormone, are the probable mediators of this effect, whereas infusions of glucose and insulin inhibit splanchnic (hepatic) clearance of amino acids (43). Tews et al. (44) showed that high protein diets also apparently increase liver capacity to transport amino acids, an increase attributed to the effects of glucagon.

Competition among similar amino acids (e.g., neutral) at physiological levels is insignificant in the liver. For example, if the perfused liver is exposed to 15 mM valine, (approximately 100 times the usual plasma concentration), the intracellular valine concentration in the liver approaches 15 mM. The concentration of other large neutral amino acids in liver remains unaltered, however (50). Concentrations of individual amino acids as high as 60 mM are necessary to inhibit the transport of neutral amino acids (for example) such as phenylalanine (51). All data thus suggests that aspartame ingestion would not inhibit amino acid transport in the liver.

Brain and Blood-brain Barrier. The association of hyperphenylalaninemia with mental retardation in PKU subjects has caused concern about the relationship between plasma levels of free amino acids and brain function. Uptake of free amino acids into the brain is dependent upon the same interactions between carrier mechanisms observed in other tissues (52, 53). One transport class includes the branched-chain and the aromatic amino acids (54). As a consequence of this common transport system, plasma levels of tyrosine, tryptophan, and the branched-chain

amino acids will influence entry of phenylalanine, and likewise a high level of phenylalanine will result in a depressed uptake of these other amino acids. Peng et al. (55) added 5% L-phenylalanine to the diet of the rat, which resulted in a seven- and eight-fold elevation of plasma phenylalanine and tyrosine levels, and a four-fold elevation in brain levels. The brain concentrations of leucine, isoleucine, valine, methionine and histidine, which occurred simultaneously, were severely depressed. This depression took place without a corresponding reduction in blood plasma levels and therefore suggested competition by phenylalanine and tyrosine for the transport carrier into the brain.

Great excesses of amino acids in the diet can alter the brain free amino acid pattern and thus protein synthesis. One good example of this effect was demonstrated by work with phenylalanine (56). Single doses of 1 g/kg phenylalanine were intraperitoneally injected into the brain of a 7-day-old rat, resulting in an increase of brain phenylalanine and a depletion of brain tryptophan. Inhibition of protein synthesis occurred and was correlated with the tryptophan depletion but not the phenylalanine increase, suggesting that the altered protein synthesis was due to lack of tryptophan. Synthesis returned to normal levels when injections of phenylalanine and tryptophan were given simultaneously.

Many studies have shown that excessive doses of other amino acids also decrease protein synthesis in the brain (57). Doses such as these do not necessarily affect brain protein synthesis by limiting uptake and availability of other amino acids. Two amino acids, L-dihydroxyphenylalanine (dopa) and L-5-hydroxytryptophan (5-HT) (58-60) cause disaggregation of whole brain polysomes by producing their respective neurotransmitter products, dopamine and serotonin. A reduction in the brain protein synthesis rate accompanies the disaggregation (61). In both

cases, the amino acid precursors (phenylalanine and tryptophan) do not inhibit protein synthesis via direct action of the amino acids themselves or from an amino acid imbalance; the polysome changes take place only after dopamine or serotonin synthesis has been completed.

As Table 1 shows, phenylalanine, as a large neutral (essential) amino acid, is preferentially transported by the L-system, which is sodium independent and does not concentrate amino acids within the cell (62). A study of phenylalanine transport in rat cerebral cortex slices (63), however, indicated a partial sodium dependency. Thus, two different transport systems for phenylalanine, one sodium-dependent and the other sodium independent, may exist in the brain.

Both dicarboxylic amino acids are thought to serve as neurotransmitters in the central nervous system. Considerable evidence suggests that L-glutamate and L-aspartate function as excitatory transmitters (64). A high affinity uptake system into nerve terminals is the physiological mechanism by which their neurotransmitter action is terminated (65, 66).

Maintenance of adequate rates of neurotransmitters and proteins in the brain relies on the availability of sufficient amounts of precursor amino acids. The rates at which amino acids enter neurons are dependent upon the affinities and capacities of transport systems for these amino acids in neuronal membranes. Reports that changes in brain tryptophan, tyrosine, and phenylalanine pools alter the synthesis of proteins and neurotransmitters led to a study by Archer and colleagues (67). Amino acid transport was studied in three neuroblastoma clones: N-TD6, which synthesizes norepinephrine; N-T16, which synthesizes small amounts of serotonin; and N-S20Y, which synthesizes acetylcholine. Tyrosine, phenylalanine and tryptophan enter all three clones by rapidly exchanging trans-

port systems. Results of this study suggested that if similar amino acid transport systems exist in neuronal membranes and if amino acid levels in brain extracellular fluid are similar to levels in plasma, such systems may serve to limit the entry of amino acids into brain cells when blood amino acid levels are near the normal physiological range. Those results thus lead to the conclusion that amino acid pools in the brain are controlled by neuronal amino acid transport systems and are therefore limited so that neurotransmitter synthesis is not altered.

Muscle. Degradation of amino acids in the muscle is mainly limited to the branched chain amino acids (68). Other amino acids, including aspartate and phenylalanine, are taken up and degraded in relatively small amounts by the muscle. However, the supply of those amino acids to muscle may be enhanced by adaptive increases in transport as well as by increases in plasma amino acid levels. (Such increases may occur as adaptations to exercise and will be discussed separately in a later section).

Studies of amino acid exchange across muscle tissue of the human forearm have revealed that in the postabsorptive state, there is a net release of amino acids from muscle (69-71). The output of alanine and glutamine was greater than that for all other amino acids and was responsible for over 50% of total alpha amino nitrogen release (72).

Net uptake during rest consists of small but consistent uptakes of serine, cystine, and glutamate (73-75). Aspartic acid is taken up by resting muscle also, but in comparatively insignificant amounts, as Ahlborg et al. (76) demonstrated. Phenylalanine was shown to have a net release from resting muscle, although the amounts released were also found to be relatively small (table 2, next section).

Competition between neutral and aromatic amino acids (e.g. phenylalanine) for uptake is not a problem in muscle. Guoff et al. (77) demonstrated that fact by a study in which pharmacological doses of aromatic amino acids markedly inhibited the brain uptake of other neutral amino acids yet failed to affect muscle amino acid levels. Christensen and Cullen (78) showed that large intraperitoneal doses (1000 mg/kg) of AIB (the nonmetabolizable amino acid α-aminoisobutyric acid) raised plasma AIB levels to 15 mM yet exerted no competitive effects on muscle uptake of other circulating amino acids.

In general, studies on muscle amino acid transport provided evidence which indicated that phenylalanine transport as well as that of other neutral amino acids is unaffected by the presence of other amino acids. All evidence gathered thus far suggests that at physiological plasma amino acid levels, competition for muscle transport sites does not occur.

Hormone Interactions

Insulin. Amino acid metabolism and, consequently, plasma amino acid levels, are affected by hormones, the most pronounced effect being exerted by insulin (79). Deposition of plasma amino acids in muscle due to insulin secretion can be stimulated by carbohydrate in the diet. This effect results in a depression of plasma levels of many amino acids, especially the branched-chain amino acids, (valine, leucine, isoleucine), phenylalanine and methionine (80-83). Therefore, free amino acid levels in the blood after a meal are dependent upon the quantity of ingested amino acids removed by the liver and upon the amount deposited in the peripheral tissues, notably in muscle, as a result of the action of insulin. In addition to its effect in decreasing the concentration of circulating amino acids, insulin also influences interorgan exchange of amino acids and is related to gluconeogenesis as well.

Felig (84) demonstrated that insulin lowered the concentrations of amino acids effectively in eviscerated animals, which suggested that muscle tissue was the site of insulin-mediated amino acid uptake. Lotspetch (85) showed that dogs responded to insulin by a similar lowering of individual amino acids which corresponded to their relative concentrations in muscle protein. Studies in the human forearm have demonstrated that the insulin effect of decreasing plasma amino acid levels is due to a net inhibition of amino acid output from muscle (86). A reduction in net output was observed for leucine, isoleucine, methionine, tyrosine, phenylalanine, and threonine. A net uptake of glutamate was demonstrated (87).

Contrary to the effect noted in other amino acids, insulin secretion causes a rise in arterial alanine levels in normal man (88). Insulin effects on lowering alanine plasma levels or inhibiting muscle output of alanine have been absent. In isolated diaphragm, insulin has led to an increased alanine output from muscle while leading to a decrease in the release of all other amino acids (89). This behavior can be attributed to insulin-stimulated synthesis of alanine from glucose-derived pyruvate, as proposed in the glucose-alanine cycle (90).

Insulin also exerts its influence on amino acid metabolism via its role in the regulation of gluconeogenesis. Not only does insulin increase glucose utilization by fat and muscle, but it also inhibits glucose release from the liver (91). Direct evidence of this effect was provided by a study demonstrating insulin inhibition of ^{14}C-alanine incorporation into glucose in the perfused liver (92).

Glucagon. Pharmacologic doses of glucagon exert a hypoaminoacidemic effect on virtually all amino acids (93-95). In contrast to the effects imposed by insulin, alanine is the amino acid most affected by glucagon, and it shows the greatest decline in plasma levels (96). Increased uptake by the liver has been observed to be responsible for that effect (97). In the perfused liver, glucagon enhances the conversion of alanine to glucose (98) and stimulates intracellular transport and utilization of glycogenic amino acids (99), also an effect most pronounced in alanine.

In muscle, glucagon increases branched chain amino acid oxidation (100), decreases protein synthesis from amino acids (101), and increases muscle amino acid output (102). In general, glucagon exhibits a catabolic effect on amino acid metabolism in which the transfer of amino acids from muscle to liver for conversion to glucose occurs.

Amino Acids as Secretagogues. Hormone-amino acid interactions not only include the effects of hormones on amino acid metabolism, but also the reverse effects --- amino acid stimulation of hormone secretion. Protein ingestion may in fact be the most important physiologic stimulus for glucagon secretion, a major factor in the prevention of hypoglycemia which otherwise would accompany the insulin increase after a protein meal (103).

Aspartic acid and phenylalanine are major glucagon secretogogues as well as strong insulin β-cell secretogogues (104, 105). Studies thus far, however, have indicated that aspartate or phenylalanine ingested in the form of aspartame do not stimulate either glucagon or insulin secretion (106, 107).

III. Aspartame and Exercise

In considering the total population of weight-conscious Americans, an increasing number of this total are utilizing exercise as a method of weight control. Although no formal statistics have been accumulated, it is reasonable to assume that many of these "exercisers" also incorporate the use of the low calorie sweetener aspartame (4 kcal/gram) in their diets as an added measure of weight control. It is therefore important to determine the physiological effects of interaction between aspartame and exercise.

Since studies on this specific interaction are virtually nonexistent, protein metabolism in exercise will be focused upon. Any specific effects of aspartame or its component amino acids will be noted.

Composition of Muscle. Muscle tissue accounts for approximately 40% of total body weight, representing the largest source of protein in the body (108). The most predominant type of muscle, the skeletal or striated muscle, is that which is directly responsible for performing work during exercise. Skeletal muscle is composed of 75% water, 20% protein, and 5% inorganic salts and other substances. The most abundant muscular proteins are myosin, actin and tropomyosin, comprising about 52%, 23% and 15%, respectively of the total protein content of muscle. Also, the conjugated protein myoglobin is incorporated into muscle tissue (700 mg Mb/100 g muscle tissue) (109).

Due to the contribution of protein to muscle composition, it is evident that protein is important in the structure and function of muscle. But how important is protein as an energy source? Does pro-

tein supply a significant amount of fuel during exercise? These are questions that must be considered in determining the effects of interaction between aspartame and exercise.

Protein Metabolism and Exercise. Energy for muscle contraction is derived through hydrolysis of the high-energy phosphate bonds from either adenosine triphosphate (ATP) or creatine phosphate. Due to the small concentration of these substances in muscle, a continual regeneration of the high energy bonds must occur if exercise is to proceed for an extended length of time. The role of skeletal muscle in exercise is to regenerate ATP from anaerobic and oxidative metabolism of carbohydrate and fat. Together, these two fuels provide approximately 90% of the energy required for exercise (110, 111). Thus, the contribution of protein to the fuel supply used during exercise is relatively minor in comparison to that of carbohydrate and fat. However, current research has revealed that amino acids can contribute to whole body metabolism, providing up to 5.5% of the total caloric cost of exercise, as estimated by Evans et al. (112). Two major approaches in the determination of the amount of protein used as fuel during exercise are 1) nitrogen excretion studies and 2) amino acid oxidation studies. Results of those studies will help clarify the role of protein as an energy source.

Nitrogen Excretion Studies. Decombaz (113) measured urinary urea clearance in 11 trained runners after completing a 100 km race, recording a net decrease in rates of urea clearance during the race and a net increase during the 24-hr period following. Plasma urea levels were 50% higher after the run, remaining elevated for 24 hours. The rise in plasma urea accounted for only a portion of the decline in urea

clearance rates; urea production rates averaged 44% higher during the race in comparison to pre-race rates. In a similar study (114), neither a decline in urea clearance during a 2 hour bicycle ergometer ride or during a 70 km ski race could explain the rise in plasma urea.

Those studies show that continuous moderate to heavy exercise increases urea production and decreases urea clearance that is approximately balanced by an increased elimination of urea through sweat. Based on those results, Lemon and Mullin (115) estimated that up to 12% of the energy cost of exercise may be contributed by protein.

Amino Acid Oxidation Studies. The second method used to determine the amount of protein used as fuel during exercise is the measurement of the oxidation of amino acids. Until recently, the liver was believed to be the major location of amino acid degradation in mammals (116). However, several studies have now proven that non-hepatic tissues, especially skeletal muscle, can oxidize amino acids, the branched chain amino acids (BCAA) being preferred (117-119). Khatra et al. (120) showed that 60% of the total body distribution of the enzymes necessary for BCAA catabolism (BC α-keto acid dehydrogenases) are located in skeletal muscle. Another study (121) demonstrated that the oxidation of alanine, glutamate, and leucine may contribute up to 20% of the total CO_2 produced when isolated muscle is supplied with a medium of 18 amino acids (and no other substrates). The oxidation of valine, isoleucine, and aspartate also has been reported (122) in skeletal muscle. Dohm et al. (123) found that leucine oxication increased with endurance training, suggesting that enzymes involved in muscle leucine oxidation may undergo an adaptation to training similar to that observed in carbohydrate and fat catabolism enzymes.

Some variation in BCAA oxidation occurs among species, as the total BC α-keto acid dehydrogenases in rat muscle is significantly less than that in man. (10% in rat; 60% in man) (124) Therefore, BCAA oxidation in human skeletal muscle is much more likely than in the rat. The studies cited above were performed on rats, but it is possible that amino acid oxidation in man makes a significantly larger contribution to exercise.

The Glucose-Alanine Cycle. Felig et al. (125) observed an increased alanine output from exercising skeletal muscle and in another study (126) demonstrated that this rise in alanine output is proportional to the intensity of exercise. Those results suggested that amino acids in muscle are transaminated to form alanine from glucose-derived pyruvate (Fig. 6). The source of the amino groups for the formation of alanine may be the BCAA within skeletal muscle (127). That theory was supported by the observation that alanine released from exercising muscle increased when leucine was available via the bathing medium.

Another important factor is the release of liver BCAA into the blood, unlike other amino acids which can be utilized for gluconeogenesis. That effect suggested that the liver also may provide BCAA to skeletal muscle (128). Shunting those amino acids to muscle would supply an additional source of carbon to be oxidized for energy. Decreased insulin and increased glucagon during exercise would enhance protein degradation and thus maximize the supply of BCAA to be oxidized (129). In combination with the enhanced glucose utilization during exercise, that fact could account for the dramatic increase in alanine output from working skeletal muscle.

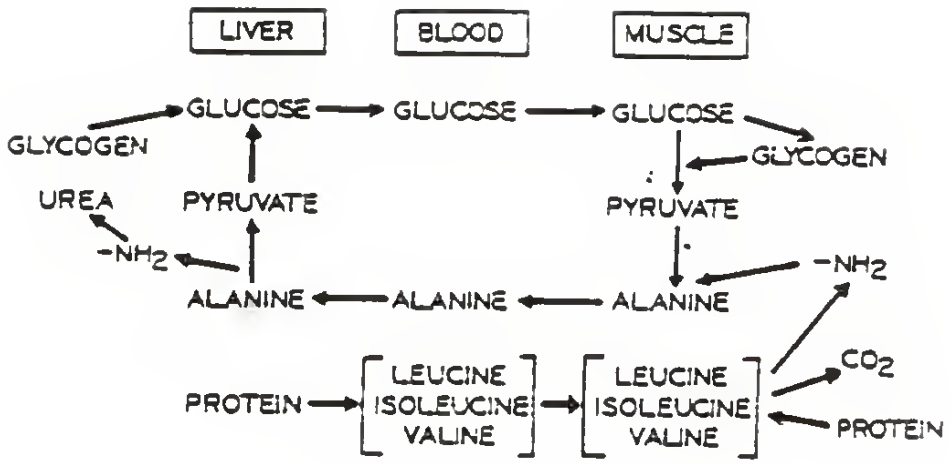


Fig. 6 The glucose-alanine cycle. Odessey et al. (127).

Ahlborg et al. (130) determined the net release and uptake of amino acids in leg muscles before and during exercise. In addition to the net uptake of BCAA as observed in the previously cited studies, results of phenylalanine and aspartate exchange also were noted. The exercised muscle showed a net uptake of both phenylalanine and aspartate, although not in significant amounts in comparison to the quantity of BCAA taken up (table 2).

Glucoregulatory Hormones and Exercise. Another effect of exercise which may indirectly affect amino acid transport is the stimulation of increased glucagon to meet increased energy needs of muscle contraction. Chambers et al. (131) demonstrated that glucagon increased amino acid uptake by the isolated rat liver when administered *in vivo* prior to sacrifice or when added directly to the isolated system. Glucagon stimulation by exercise also caused increased lipolysis and therefore an increase in intracellular free fatty acids. Herrera and Renold (132) suggested that those fatty acids played a role in regulating amino acid uptake through uncoupling oxidative phosphorylation and thus, in decreasing the supply of energy for active transport. Insulin could release this inhibitory effect on transport by decreasing lipolysis. The evidence from that study suggested decreased insulin levels due to exercise may inhibit amino acid transport in the muscle. That may be necessary for the increased uptake of most amino acids (including phenylalanine and aspartate) during exercise.

Other studies indicated that the physiologic importance of hormone changes during exercise relate primarily to stimulation of hepatic glucose production rather than enhancement of glucose utilization (133).

Table 2 Arterial concentrations, splanchnic exchange, and leg exchange of individual neutral and acidic plasma amino acids at rest and during prolonged exercise. Ahlborg et al. (130).

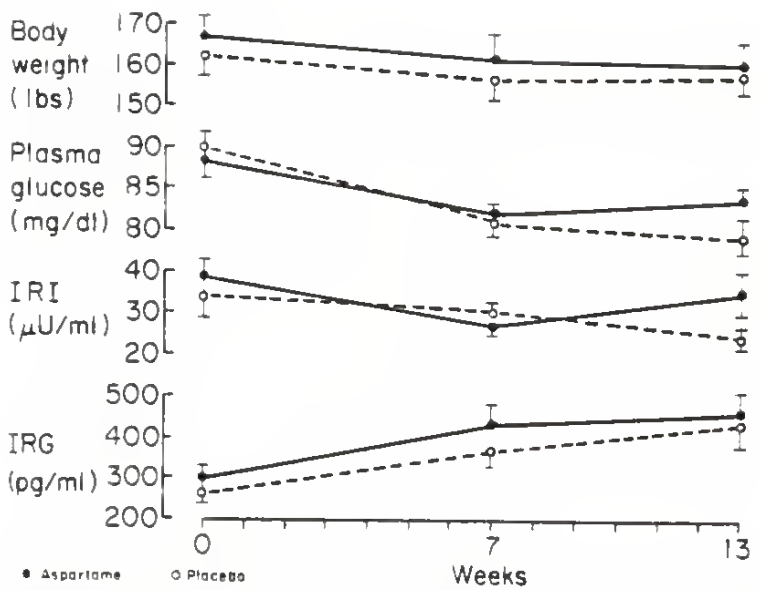
	Arterial concentration			Splanchnic exchange			Leg exchange‡		
		Exercise			Exercise			Exercise	
	Rest	40 min	240 min	Rest	40 min	240 min	Rest	40 min	240 min
	μmol/liter			*μmol/min*			*μmol/min*		
Taurine	43±4	46±3	41±1	1.9±0.4	1.2±1.5	−1.6±2.5	1.4±1.0	−9.8±7.0	4.2±6.4
Aspartate	18±2	21±4	16±2	−1.0±1.5	2.7±3.1	2.8±0.7	−1.8±1.0	1.8±4.6	7.2±4.6
Threonine	112±15	114±14	85±16§	10.1±1.5	10.8±1.1	9.9±6.0	−4.0±2.2	2.2±4.4	−14.6±20.4
Serine	119±10	133±18	99±9§	16.2±5.0	27.2±15.9	22.4±4.2	5.4±2.0	12.2±21.0	17.0±5.4
Proline	172±10	174±13	131±9	5.7±2.5	8.9±4.8	15.2±2.7§	−4.8±5.6	15.8±22.6	2.2±31.8
Citrulline	36±3	36±2	37±3	−8.5±0.8	−13.1±3.1	−8.5±1.9	2.0±0.6	−4.8±6.8	10.2±2.4‖
Glycine	188±16	206±11	160±8	8.3±2.2	25.2±11.2	31.8±4.6¶	−8.0±4.8	7.6±15.2	17.8±17.4
Alanine	192±22	271±29¶	233±30	57.6±4.3	89.7±21.4	119.0±18.9¶	−80.4±7.6	−44.6±20.0	−95.4±44.6§
α-Aminobutyrate	29±1	26±2	22±1§	−3.8±3.6	−2.2±1.9	13.8±11.2	0.2±0.4	3.4±3.8	5.8±4.4
Valine	242±13	238±11	243±9	−3.2±1.5	−9.1±6.0	−31.6±6.8¶	0.6±3.8	31.6±17.0	43.4±15.6‖
Cystine	84±15	92±11	102±12	3.5±1.3	−1.3±6.0	5.7±4.2	6.2±2.6	35.2±16.0	23.6±20.8
Methionine	17±2	19±2	18±1	1.4±0.6	2.6±1.5	4.9±1.2	−0.6±0.4	−2.8±2.6	−4.0±4.0
Isoleucine	60±4	60±2	81±6	−1.0±0.8	−2.9±2.5	−17.2±2.9¶	−0.4±1.2	8.2±8.0	21.8±4.2¶
Leucine	126±7	127±4	151±10§	−2.2±0.8	−8.8±3.5	−30.2±4.7¶	−0.8±2.0	13.2±10.4	28.6±7.8¶
Tyrosine	45±4	49±5	59±4	5.1±0.3	4.7±1.0	6.5±1.4	−1.2±0.8	9.0±7.6	8.4±9.4
Phenylalanine	44±3	49±3	55±4§	2.4±0.5	3.0±1.1	4.1±2.5	−1.0±0.6	7.6±7.6	5.2±9.0

* Data are presented as mean±SEM.
‡ Data refer to amino acid exchange across both legs.
‖ Significantly different from the corresponding value at rest, $P < 0.05$ (paired t test).
¶ Significantly different from the corresponding value at rest, $P < 0.02$ (paired t test).
# Significantly different from the corresponding value at rest, $P < 0.005$ (paired t test).

Those studies have demonstrated the extreme sensitivity of liver glycogenolysis to the inhibition exerted by insulin, thus stressing the importance of hypoinsulinemia in regulating hepatic glucose production. In prolonged or severe exercise, increased glucagon and growth hormone also contribute to this response. However, neither hypoinsulinemia nor hyperglucagonemia have been proven necessary for exercise-induced stimulation of liver glucose output (134).

Effects of Aspartame During Weight Reduction. Because a potent sweetener of little caloric value such as aspartame is very likely to be used by weight-conscious persons, Knopp et al. (135) decided to determine the potential for aspartame toxicity during weight reduction. The possibility of long-term effects of aspartame on the fuel hormonal alterations characteristic of calorie restriction were the focus in their study. Fig. 7 illustrates the changes in plasma immunoreactive insulin (IRI) and glucagon (IRG) as well as the extent of the weight reduction and glucose reduction which occurred in both aspartame and placebo groups. In general, the results suggested that weight reduction in the subjects studied caused a detectable shift in metabolism, while simultaneous administration of aspartame did not result in a significant effect. The changes in IRI and IRG at week 7 were due to the decreased food intake and the increased dependence on endogenous fuels for energy and the need for enhanced gluconeogenesis to meet the needs of the brain and other tissues, such as muscle (136).

Although shifts in glucose, insulin and glucagon normally accompany caloric restriction (137), the study by Knopp et al. (135) indicated that aspartame produced no significant effects during weight reduction. Be-



Effect of ingestion of aspartame (A) or placebo (P) on body weight, plasma glucose, immunoreactive insulin (IRI), and immunoreactive glucagon (IRG) during caloric restriction in young persons. At no time are A and P significantly different from each other. Within A and P groups paired comparisons against the initial measurement (week 0) show significant differences at weeks 7 and 13. Significant reductions are seen in body weight at both weeks 7 and 13 ($p < 0.001$ for A and P in every instance). For glucose at week 7, significant reductions are seen in A ($p < 0.01$) and P ($p < 0.05$). At week 13 glucose remains significantly reduced only in P ($p < 0.001$). IRI is significantly reduced only at week 7, A ($p < 0.025$), P ($p < 0.01$). For IRG, significant elevations are seen at week 7 ($p < 0.001$ for both A and P) and at week 13 A ($p < 0.01$) and P ($p < 0.001$).

Fig. 7 Effect of aspartame on insulin and glucagon during caloric restriction in young persons. (135)

cause both aspartic acid and phenylalanine, as mentioned previously, are potent glucagon secretogogues, it is important that those particular amino acids ingested in the form of aspartame do not exhibit synergistic effects with another glucagon stimulus such as calorie restriction. If ingestion of aspartame shows a similar non-stimulatory effect on glucagon in future studies involving weight reduction, it may be reasonable to conclude that aspartame ingestion might likewise have no detectable effects on exercise, since the response of glucoregulatory hormones during exercise is similar to that observed in calorie restriction.

MATERIALS AND METHODS

Animals and Their Care

The experimental animals were 3 week-old male Wistar rats (HSD: WI:BR, Harlan Sprague Dawley, Indianapolis, IN) weighing 60-8S g. Animals were housed individually in stainless steel, wire bottom cages (18 x 24 x 18 cm) and received water ad libitum throughout the nine-week study. Room conditions were maintained at 22 C with an automatic 12-h light-dark cycle.

The experimental design was a 2 x 2 factorial where the main treatment effects were aspartame supplementation[1] and exercise. The control diet (Table 2) was Teklad TD 83448 Purified Diet with 6S% cornstarch (Teklad Test Diets, Madison, WI). The aspartame-supplemented diet was formulated by substituting 0.6% aspartame for 0.6% cornstarch. Diets were isocaloric. Rats in exercise groups were exercised 7S min/day, S days/wk (Mon-Fri), on a motor-driven zero-grade treadmill[2] in the late morning hours. The initial two weeks of the nine-week study were the "training" period in which the animals were gradually adapted to a target speed of 24 meters per minute (Table 3). This speed was maintained for the remaining seven weeks of the study.

Rats were weighed twice a week (Mon. and Sat.) and feed intake was measured daily throughout the nine-week period. Weekday weight gain was calculated by subtracting Monday body weight from Saturday body weight. Weekend weight gain was calculated by subtracting the previous Saturday body weight from the Monday body weight. Daily feed intake for each rat was derived by subtracting amount of feed left or spilled each day from weight of feed administered on the previous day.

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[1]The aspartame was generously supplied by the Searle Company (Skokie, Ill.) Correspondence is in appendix tables 1 and 2.

[2]Radiotrol Treadmill, ½ h.p., Boston Gears, Quincy, MA.

TABLE 2
Experimental diets

	Control[1]	Aspartame Supplemented[2]
	%	
Casein, high protein	20.0	20.0
DL-Methionine	0.3	0.3
Corn Starch	65.0	64.4
Corn Oil	5.0	5.0
Fiber (cellulose)	5.0	5.0
Mineral Mix, AIN-76	3.5	3.5
Vitamin Mix, AIN-76A	1.0	1.0
Choline Bitartrate	0.2	0.2
Ethoxyquin (antioxidant)	.001	.001
Aspartame	0.0	0.6

[1]Teklad TD 83448, Purified Diet with 85% Corn Starch. This diet is similar to AIN-76A except that sucrose is substituted by cornstarch.

[2]Teklad TD 85033, Basal Mix (Adjusted Corn Starch). This basal mix was designed to be used at the rate of 994.0 g/kg of diet, in conjunction with 6.0 g/kg aspartame.

TABLE 3
Initial two-week training period for rats

	Treadmill dial	Meters per minute
Mon.	25	6
Tues.	35	10
Wed.	40	12
Thurs.	45	14
Fri.	50	16
Mon.	55 - 60	18
Tues.	60 - 65	20
Wed.	65 - 68	22
Thurs.	70	24
Fri.	72	24

Sacrifice Procedures

At the end of the nine-week study the rats were fasted 12-16 h before being sacrificed by exsanquination through heart puncture. A dehairing mixture used previously by Crews et al. (138) was then applied to remove the rat fur. The mixture was composed of barium sulfide (200 g), Tide detergent (50 g), and 10% glycerol (500 ml mixture).

After washing the rats, the gastrointestinal tract from the gastroesophageal sphincter to the anus was removed from each rat. This measure was followed to minimize variations in body weight caused by periodic feeding behavior of the rats. The lungs also were removed to permit accurate hydrostatic (underwater) weighing. Heads and tails were removed using an electric knife. Each carcass was then blotted with paper towels (inside and out) and weighed on a Toledo dual-pan balance. This weight, for later reference, is considered "carcass weight in air."

Each carcass (whole rat minus hair, gastrointestinal tract, head and tail) was then weighed underwater. The hydrostatic weighing apparatus utilized was a simple set-up constructed as follows. A trip balance (Cent-o-gram Model 311, Ohaus) was positioned above a cylindrical glass container (22 cm diam., 31 cm ht.). The weighing pan of the scale was replaced with a commercial wire-mesh vegetable basket (Walmart) which was large enough for a rat carcass but small enough to fit inside the glass cylinder. The wire-mesh basket was weighted with ordinary fishing sinkers to provide additional gravity, but an no time during the weighing procedure was the basket allowed to touch the side or bottom of the glass container. The weight of the basket alone was recorded prior to weighing each carcass. Water temperature also was noted for each weighing to cal-

culate the K value for the Specific Gravity equation later described. Appendix Table 3 shows the relationship between temperature and water density.

The carcass was submerged underwater in the basket and rotated to release any air bubbles trapped in the body cavity, and the skin was then stroked to remove any additional clinging air bubbles. Weight was recorded to the nearest .01 g.

Body specific gravity was calculated using the following formula, used previously be Dahms and Glass (142) which showed a strong correlation between percent body fat and body specific gravity in rats:

$$\text{Specific Gravity} = \frac{WA}{(WA - WW)} \cdot K$$

WA = carcass weight in air
WW = carcass weight in water
K = specific gravity of H_2O saturated with air (.997327 @ 24 C)

Percent body fat was then calculated as follows:

$$\% \text{ fat} = -394.498(\text{specific gravity}) + 431.35$$

Upon completion of the hydrostatic weighing procedure, the liver, kidneys and heart were removed from each carcass and weighed to the nearest .01 g. The organs were then blotted dry and wrapped individually in labelled foil wrappers for storage at -18 C.

Next, abdominal, epididymal, and retroperitoneal fat deposits were removed and weighed to the nearest .1 g. These tissues then were discarded. Finally, four leg muscles were removed from the left hind limb of each carcass: the gastrocnemius lateralis and medialis as well as the vastus lateralis and medialis. The vastus muscles were from the thigh; the gastroc muscles from the calf. The muscles were weighed to the nearest .01 g, wrapped, and stored as described for the tissues.

The blood samples (8-10 ml each) that were taken by cardiac puncture were allowed to clot (30-60 s) and immediately centrifuged at 5000 g for 8 min to separate the serum. Serum was stored at -18 C until later analysis.

Analytical Procedures

Serum triglycerides. Serum triglycerides were measured enzymatically using Fisher Kit No. CS-876 (Fisher Scientific, Fair Lawn, NJ). In this procedure triglycerides are hydrolyzed to free fatty acids and glycerol. The glycerol is converted to dihydroxyacetone phosphate through a series of steps where $NADH_2$ is released and forms formazan. The latter is a pink color readable at 500 nm.

The first step of the assay procedure was the reconstitution of triglyceride reagent vial (CS-876-A) with 11.0 ml buffer (CS-876-B). Two ml aliquots of triglyceride reagent were placed in 12 x 77 ml disposable tubes and warmed in a 37 C water bath for 5 minutes. At timed 30 s intervals 20 µl of either saline (blank), standard (200 mg% triolein), or serum were pipetted into the tubes, mixed, and allowed to incubate 10 min. Usually each run included one blank, one standard and ten samples. At the end of the 10 min incubation, tubes were removed again at 30-second intervals and absorbance was read with a Brinkmann PC 800 colorimeter. The latter instrument had a 545 nm filter which, even though it was not the 500 nm suggested, previous studies indicated it was suitable for measuring pink chromophore reactions. Triglyceride contents of each serum sample were determined by linear regression using the blank and standard values as reference points.

Serum cholesterol. Total, HDL, and LDL cholesterol levels were measured using the LDL-Direct Cholesterol Audit System (Isolab, Akron, OH). In this assay total cholesterol was determined by adding serum directly to the cholesterol reagent; but for HDL and LDL cholesterol the serum was added to an affinity column, eluted sequentially with Alpha Agent for Alpha cholesterol, and Beta Agent for HDL cholesterol. The fractions collected were then added to cholesterol reagent, mixed, and incubated at 37 C for 15 min. The absorbance of the pink chromophore was read at 545 nm with a Brinkman PC 800 colorimeter. Specific instructions on column preparation and procedures are in Appendix Table 4 and are adapted from those given by another investigator (139).

Serum glucose. Glucose was determined using Sigma Kit No. 510 (Sigma Diagnostics, St. Louis, MO), which is a quantitative, enzymatic (glucose oxidase) procedure. In this procedure serum is added to a mixture of glucose oxidase, peroxidase, and o-dianisidine. During incubation the glucose is converted to gluconic acid and H_2O_2. The hydrogen peroxide then reacts with the o-dianisidine to form oxidized o-dianisidine, a brown chromophore.

In this procedure the PGO enzymes and o-dianisidine are reconstituted to form the combined color reagent. Five tenths ml sample (diluted 1:20) or standard (100 mg/dl diluted 1:20) or water (blank) was added to 5.0 ml combined color reagent. After mixing tubes were incubated at 37 C for 30 min for the brown color development, samples were read on a Summerson Klett meter using a green filter. Glucose contents of each serum sample were determined by linear regression using the blank and standard values as reference points.

Tissue lipids. Tissue lipid levels were determined for liver, kidneys (both pooled), heart, and muscles taken from the left hindlimb including vastus lateralis, vastus medialis, gastrocnemius lateralis, and gastrocnemius medialis. The Folch gravimetric method (140) was used except that the initial extraction mixture was methylene chloride:methanol (2:1, v/v) as suggested by Chen et al (141) instead of the more hazardous chloroform:methanol mixture used by Folch.

Aluminum dishes (S/P catalog No. D2165) were labeled with a permanent marking pen, dried 1-h in a 103 C forced-air draft oven, cooled at least 1-h in a desiccator, and weighed to the nearest mg. Dishes were then arranged in the work area so that pipetting could be performed in an organized manner.

After the dishes had been prepared, samples weighing 0.5 - 1.5 g were homogenized in 15 ml methylene chloride:methanol for 30 s using a Polytron high-speed homogenizer (Brinkmann Instruments, Westburg, NY) which worked well even for the muscle tissues. Homogenates were then filtered, and collected matter was washed with 5 additional ml methylene chloride: methanol. Four ml 0.73% NaCl were added and tubes were shaken 5 min on an automatic shaker. After centrifugation for 5 min the top aqueous layer was aspirated off and 7.0 ml of the bottom organic layer (containing the lipids) was pipetted into the aluminum dishes. The solvent was allowed to evaporate and the dishes were again dried at 103 C for 1 h. After cooling in a desiccator, dishes containing the lipid residue were weighed to the nearest mg. Percent fat (lipid) was calculated by multiplying mg lipid in each dish by 16/7 (because 7.0 ml organic layer was used out of a total of 16), dividing by weight (in grams), dividing by 1000 (to convert mg to g), and multiplying by 100 (to get %).

Statistical Analyses.

Two-way analysis of variance procedures were used where the main treatment effects were exercise and aspartame. The interaction between exercise and aspartame was also examined. The sources of variation are shown below:

SOURCE	df
Model	(3)
Exercise	1
Aspartame	1
Exercise*Aspartame	1
Error	32
Corrected Total	35

Analysis for each dependent variable was accomplished using SAS (Statistical Analysis System) computer language. A copy of the program is shown in Appendix table 5.

RESULTS

Effects of Aspartame and Exercise on Weight Gain

The effects of aspartame and exercise on weight gain of rats during the nine week study are presented in Table 4. When total weight gain of all groups was compared, exercised animals showed a significantly lower weight gain ($P<.01$) than the non-exercised groups. When average daily gain of all groups was compared, the exercised animals again showed a significantly lower weight gain ($P<.01$). These effects were attributed to the lower average weekday gain (M-F), as no significant differences among groups were detected in average weekend gain. Aspartame-fed rats gained a similar amount of weight when compared to those not fed aspartame.

Effects of Aspartame and Exercise on Feed Intake

The effects of aspartame and exercise on feed intake during the nine week study are shown in Table 5. The average daily feed consumption of exercised animals was significantly lower ($P<.01$) than that of non-exercised animals, even when expressed per 100g body weight. No significant effects on total daily feed intake resulted from aspartame supplementation. However, when average daily feed consumption per 100g body weight was calculated, aspartame supplementation did have significant effects on daily feed intake. Both aspartame-supplemented groups (non-exercised and exercised rats) consumed less feed per 100g body weight ($P<.05$) than those not fed aspartame.

The lower feed consumption of exercised rats was attributable to average weekday feed consumption rather than average weekend feed consumption. The exercised animals consumed less feed per weekday ($P<.01$)

TABLE 4
Effects of aspartame and exercise on weight gain of growing male rats*

Measurement	-Exercise		+Exercise		ANOVA, effects		
	-Aspartame	+Aspartame	-Aspartame	+Aspartame	Exer	Aspart	Exer*Aspart
	g						
Initial weight	69 ±5	70 ±4	72 ±5	69 ±4	NS	NS	NS
Final weight	439 ±11	447 ±14	412 ±8	420 ±9	0.01	NS	NS
Weight gain	371 ±9	377 ±13	340 ±7	351 ±11	0.01	NS	NS
Average daily gain	6.1 ±.1	6.2 ±.2	5.6 ±.1	5.8 ±.2	0.01	NS	NS
Average weekday (M-F) gain	6.1 ±.2	6.2 ±.4	5.1 ±.1	5.2 ±.2	0.01	NS	NS
Average weekend (S-S) gain	6.1 ±.2	6.2 ±1.0	6.9 ±.3	7.4 ±.2	NS	NS	NS

*Values are means ± SEM for 9 rats per treatment group. Analysis of variance: significant ($P<0.05$ or 0.01) or nonsignificant (NS) effects are indicated. Individual values shown in Appendix Tables 6-7.

TABLE 5
Effects of aspartame and exercise on feed intake of growing male rats*

Measurement	-Exercise		+Exercise		ANOVA, effects		
	-Aspartame	+Aspartame	-Aspartame	+Aspartame	Exer	Aspart	Exer*Aspart
	----------g----------						
Average daily feed consumption							
Daily total	22.1 ±.5	21.4 ±.6	19.7 ±.4	19.7 ±.4	0.01	NS	NS
Per 100g body weight	9.8 ±.2	9.0 ±.2	8.8 ±.2	8.7 ±.1	0.01	0.05	NS
Average weekday (M-F) feed consumption							
Daily total	21.8 ±.5	20.5 ±.6	18.5 ±.4	18.6 ±.4	0.01	NS	NS
Per 100g body weight	10.0 ±.2	9.0 ±.2	8.6 ±.2	8.5 ±.2	0.01	0.01	0.05
Average weekend (S-S) feed consumption							
Daily total	23.0 ±.5	23.9 ±.7	22.9 ±.4	22.9 ±.5	NS	NS	NS
Per 100g body weight	9.2 ±.2	9.3 ±.2	9.5 ±.2	9.3 ±.1	NS	NS	NS

*Values are means ± SEM for 9 rats per treatment group. Analysis of variance: significant ($P<0.05$ or 0.01) or nonsignificant (NS) effects are indicated. Individual values are shown in Appendix Tables 8-10.

than rats that were not exercised, but these differences were not observed during weekends. Aspartame supplementation did not significantly affect total weekday feed consumption. However, when average weekday (M-F) feed consumption was expressed relative to body weight, aspartame groups had significantly less weekday feed consumption ($P<.01$).

An interaction between exercise and aspartame also was noted when observing feed intake per 100g body weight (Table 5). Aspartame treatment did not seem to influence feed intake in exercised rats; however, non-exercised rats fed aspartame had lower daily relative feed intakes than those not fed aspartame. (interaction, $P<.05$).

Effects of Aspartame and Exercise on Body Fat Contents

The effects of aspartame and exercise on various body fat contents of the rats studied are shown in Table 6. Exercised animals had significantly lower ($P<.01$) percent total body fat and weights of fat depots studied including epididymal fat, retroperitoneal fat, and abdominal fat. This was observed even when fat depots were expressed per 100g body weight (with the exception of retroperitoneal fat, which did not show a significant decrease in fat per 100g body weight due to exercise).

Aspartame supplementation resulted in no significant effects on body fat contents. Aspartame in combination with exercise also failed to yield any effects on fat contents.

Effects of Aspartame and Exercise on Tissue Weights and Lipid Contents

The effects of aspartame and exercise on tissue weights and lipid contents of rats in the 9 week study are shown in Table 7.

Liver. Exercised animals had smaller livers ($P<.05$) than non-exercised animals. When expressed as percent of body weight, however, no

TABLE 6
Effects of aspartame and exercise on body fat contents of growing male rats*

Measurement	-Exercise		+Exercise		ANOVA , effects		
	-Aspartame	+Aspartame	-Aspartame	+Aspartame	Exer	Aspart	Exer*Aspart
Body fat (%)	16.4 ±1.0	19.4 ±0.7	14.7 ±0.8	13.9 ±1.1	0.01	NS	NS
Epididymal fat							
Weight (g)	6.6 ±0.7	8.3 ±0.8	5.4 ±0.4	5.6 ±0.4	0.01	NS	NS
Per 100g body weight (g)	1.5 ±0.1	1.8 ±0.1	1.3 ±0.1	1.3 ±0.1	0.01	NS	NS
Retroperitoneal fat							
Weight (g)	9.1 ±1.0	11.3 ±1.0	6.1 ±0.6	5.7 ±0.6	0.01	NS	NS
Per 100g body weight (g)	2.1 ±0.2	2.5 ±0.2	1.5 ±0.1	1.4 ±0.1	0.01	NS	NS
Abdominal fat							
Weight (g)	6.7 ±0.7	7.5 ±0.7	4.4 ±0.4	4.5 ±0.4	0.01	NS	NS
Per 100g body weight (g)	1.5 ±0.1	1.7 ±0.1	1.1 ±0.1	1.1 ±0.1	0.01	NS	NS

*Values are means ± SEM for 9 rats per treatment group. Analysis of variance: significant ($P<0.05$ or 0.01) or nonsignificant (NS) are indicated. Individual values shown in Appendix Tables 11-14.

TABLE 7
Effects of aspartame and exercise on tissue weights and lipid contents in growing male rats*

Measurement	-Exercise		+Exercise		ANOVA, effects		
	-Aspartame	+Aspartame	-Aspartame	+Aspartame	Exer	Aspart	Exer*Aspart
Liver							
Weight (g)	13.72±.51	13.85±.69	12.19±.39	12.93±.38	0.05	NS	NS
Per 100g body weight (g)	3.12±.08	3.08±.07	2.95±.06	3.07±.05	NS	NS	NS
Fat (%)	12.67±1.10	10.98±.68	9.99±.83	10.89±1.06	NS	NS	NS
Kidney							
Weight (g)	2.62±.06	2.71±.07	2.73±.09	3.00±.09	0.05	0.05	NS
Per 100g body weight (g)	0.60±.01	0.61±.01	0.66±.01	0.71±.01	0.01	0.05	NS
Fat (%)	6.92±.20	6.67±.21	6.57±.08	6.29±.11	0.05	NS	NS
Heart							
Weight (g)	1.36±.04	1.34±.04	1.30±.04	1.33±.03	NS	NS	NS
Per 100g body weight (g)	0.31±.01	0.30±.01	0.32±.01	0.32±.00	NS	NS	NS
Fat (%)	4.60±.06	4.52±.10	4.51±.06	4.63±.10	NS	NS	NS

*Values are means ± SEM for 8-9 rats per treatment group. Analysis of variance: significant ($P<0.05$ or 0.01) or nonsignificant (NS) effects are indicated. Individual values shown in Appendix Tables 15-17.

treatments resulted in significant effects on liver size. Liver lipid contents also were unaffected by either aspartame or exercise.

Kidney. Both exercised rats and aspartame-supplemented rats had larger kidneys ($P<.05$) than control rats, even when considering kidney weight relative to body weight. The combination of aspartame and exercise did not have a significant effect on kidney size.

Lipid contents of kidneys were lower in exercised rats ($P<.05$) and aspartame treatment did not have any significant effect on this measurement.

Heart. Heart weight and lipid contents of heart tissue were not significantly altered by any of the experimental treatments.

Effects of Aspartame and Exercise on Muscle Weights and Lipid Contents

The effects of aspartame and exercise on weights and lipid contents of muscles are shown in Table 8.

Vastus medialis. The vastus medialis weights were not significantly affected by any of the experimental treatments. However, when muscle weights were determined as a percent of body weight, the vastus medialis of exercised animals was larger ($P<.01$) than those of non-exercised animals. Also, fat contents of the vastus medialis were lower in exercised animals.

Vastus lateralis. Exercised rats had larger vastus lateralis muscles relative to body weight ($P<.05$) and lower fat contents of these muscles ($P<.05$) than did non-exercised rats. Aspartame alone did not affect the muscle weights or lipid contents of this muscle. However, an interaction was shown between aspartame and exercise. Aspartame-fed rats had heavier vastus lateralis muscles than did rats which were not

TABLE 8
Effects of aspartame and exercise on muscle weights and lipid contents in growing male rats*

Measurement	-Exercise		+Exercise		ANOVA, effects		
	-Aspartame	+Aspartame	-Aspartame	+Aspartame	Exer	Aspart	Exer*Aspart
Vastus medialis							
Weight (g)	1.34±.04	1.24±.06	1.34±.06	1.37±.03	NS	NS	NS
Per 100g body weight (g)	0.31±.01	0.28±.01	0.33±.01	0.33±.01	0.01	NS	NS
Fat (%)	3.16±.19	3.16±.16	2.74±.12	2.61±.05	0.01	NS	NS
Vastus lateralis							
Weight (g)	1.54±.06	1.53±.03	1.45±.06	1.67±.05	NS	NS	0.05
Per 100g body weight (g)	0.35±.02	0.34±.01	0.35±.01	0.40±.01	0.05	NS	0.05
Fat (%)	2.93±.17	3.01±.14	2.68±.07	2.65±.12	0.05	NS	NS
Gastrocnemius medialis							
Weight (g)	1.08±.04	1.07±.05	1.00±.03	1.07±.03	NS	NS	NS
Per 100g body weight (g)	0.25±.01	0.24±.01	0.24±.01	0.25±.01	NS	NS	NS
Fat (%)	2.86±.12	2.89±.21	2.75±.15	2.50±.07	NS	NS	NS
Gastrocnemius lateralis							
Weight (g)	1.73±.07	1.56±.04	1.54±.05	1.62±.04	NS	NS	0.05
Per 100g body weight (g)	0.39±.01	0.35±.01	0.37±.01	0.39±.01	NS	NS	0.05
Fat (%)	2.95±.13	3.23±.12	2.83±.15	2.77±.10	0.05	NS	NS

*Values are means ± SEM for 8-9 rats per treatment group. Analysis of variance: significant ($P<0.05$ or 0.01) or nonsignificant (NS) effects are indicated. Individual values shown in Appendix Tables 18-21.

exercised. This was observed even when muscle weights were expressed on a 100g body weight basis.

Gastrocnemius medialis. None of the experimental treatments significantly affected the weights or lipid contents of the gastrocnemius medialis of the rats.

Gastrocnemius lateralis. Differences between groups for this muscle were similar to those observed for the vastus lateralis. Aspartame or exercise alone did not affect the muscle weights. Lipid contents of the gastrocnemius lateralis were lower in exercised animals ($P<.05$). A significant interaction was noted between aspartame and exercise where the non-exercised rats fed aspartame had lighter muscles than non-exercised rats not receiving aspartame. These differences also were observed when muscle weights were relative to body weight.

Effects of Aspartame and Exercise on Serum Cholesterol, Triglycerides and Glucose

The effects of aspartame and exercise on serum lipids and glucose are shown in Table 9. Serum total cholesterol, LDL cholesterol, and triglycerides were lower ($P<.05$) in exercised animals. However, aspartame did not seem to affect any of these measurements.

Glucose levels were not significantly affected in any treatment groups.

TABLE 9
Effects of aspartame and exercise on serum cholesterol, triglycerides and glucose of fasted growing male rats.

Measurement	-Exercise		+Exercise		ANOVA, effects		
	-Aspartame	+Aspartame	-Aspartame	+Aspartame	Exer	Aspart	Exer*Aspart
	mg/dl						
Cholesterol							
HDL	27 ±2	32 ±4	27 ±3	21 ±2	NS	NS	NS
LDL	43 ±3	40 ±4	36 ±2	29 ±1	0.01	NS	NS
Total	65 ±4	65 ±7	56 ±4	50 ±2	0.05	NS	NS
Triglycerides	134±23	128±18	79±11	94±18	0.05	NS	NS
Glucose	160±11	137±12	144±10	169±18	NS	NS	NS

*Values are means ± SEM for 9 rats per treatment group. Analysis of variance: significant ($P<0.05$ or 0.01) or nonsignificant (NS) effects are indicated. Individual values shown in Appendix Tables 22-23.

DISCUSSION

Exercised animals in this study gained significantly less weight than the sedentary animals. Others (143-146) also have shown that exercised rats gained less weight than sedentary controls. At least two studies (145,146) report similar findings with human subjects.

Exercise also significantly decreased feed intake even when feed intake was calculated relative to body weight. Several previous studies support these findings. Katch et al. (143) showed that exercised rats had lower feed consumption than nonexercised rats. Mayer et al. (145) observed that rats exercised for 20 min to 1 h had lower feed intakes and body weights than sedentary rats.

The lower weight gains and feed intakes were observed specifically during the weekdays when rats were exercised. Exercise thus seems to have an inhibitory effect on appetite, although perhaps temporarily, in that the suppressing effects were not observed on weekends.

Exercised animals had a lower percent body fat as well as lighter fat deposits when compared to nonexercised animals. Percent body fat was lower in exercised groups and epididymal, retroperitoneal and abdominal fat deposits also were lighter in these groups. Wahlberg et al. (144) and Crews et al. (142) also showed that exercised rats had a lower percent body fat than sedentary control animals. Both Askew et al. (147) and Oscai et al. (148) reported a reduction in epididymal fat pad weights in response to exercise. Burowrecki et al. (149) showed that exercised male rats had EFP weighing 50-55% less than control rats. A study by Lau et al. (150) showed that retroperitoneal fat was 50% lower in exercised groups in comparison to that measured in sedentary groups.

Exercise did not significantly affect lipid contents of tissues other than the kidney in this study. Kidneys of exercised rats were lower in lipid contents when compared to kidneys of rats in other treatment groups. Exercise did result in lower lipid contents of three of the four muscles studied. (Lipid contents of the gastrocnemius medialis were unaffected by exercise.)

The lower serum total cholesterol and triglyceride levels observed in exercised rats in this study are similar to data reported for exercised rodents in other studies (151-154). The LDL and HDL cholesterol levels also were determined in this study using affinity columns. However, the sum of these two fractions frequently resulted in recovery exceeding 100%, so the reliability of the assay procedure is questionable. The affinity columns were originally tested for human clinical analysis (telephone communication with Isolab, Inc., Akron, OH), and it is possible that rat serum may have substances which interfere with the assay procedure.

The reason for lower relative feed intakes by the aspartame-fed rats is not clear. Both control and aspartame supplemented diets were isocaloric. The 0.6% aspartame in the experimental diet replaced 0.6% cornstarch in the control diet. Both dietary components have approximately 4 calories per gram. A recent theory is that the taste of sweetness such as that imparted by saccharin results in a release of insulin even though no calories are provided (157). It is not known whether rats perceive sweetness the same way as humans do. However, if a sweet taste were provided continually, it is possible that hormonal responses might be altered in such a manner that the animals would handle energy differ-

ently. However this is speculative, and there is at the present time no good explanation for these data. It should be investigated further in view of the fact that aspartame is now a popular dietary aid.

In this study aspartame treatment had other effects as well as those mentioned previously. Aspartame-treated rats had larger kidneys even when expressed as a percent of body weight. This finding also was noted by Hiroyuki et al. (156)

Muscle weights also were studied. An unexpected interaction was noted between aspartame and exercise on weights of vastus lateralis (thigh) and gastrocnemius lateralis (calf) muscles. Rats which were exercised and fed aspartame had heavier vastus lateralis muscles than exercised rats not fed aspartame. However, this difference was not observed in non-exercised rats. An interaction also was noted for the gastrocnemius lateralis muscle where aspartame affected muscle weights differently depending on whether or not the rats were exercised. Aspartame-supplemented rats that were exercised had larger gastrocnemius lateralis weights than aspartame-supplemented rats that were not exercised.

The differences in muscle weights could not be attributed to differences in muscle lipid content. Muscle is primarily water, containing as its second most important component (by weight) protein. However, water and protein were not determined in this experiment.

Aspartame is still a relatively new sweetener and much is still not known about its effectiveness in weight control. There are relatively few published studies involving rodents and those that exist vary with respect to dosage, age and strain of animal used, length of trial, and manner in which the aspartame is administered.

The estimated maximum dosage for humans at the 99th percentile is 34 mg aspartame per kg body weight (5). However, rat trials have utilized much higher dietary concentrations. Yokogoshi et al. (155) administered 200 mg/kg body weight to fasted rats. Hiroyuki et al. (156) gave rats either 1000, 2000, or 4000 mg/kg body weight for 104 weeks for a toxicological study. The rats in this study were fed aspartame at a level of 0.6% of their normal diet which is approximately 500 mg/kg body weight. This level was chosen because it was a realistic concentration to feed as a part of the normal diet for a 9-week period. There are not many rat trials on which to base an "ideal" dietary level of aspartame; however, the amounts chosen were less than those employed in the toxicity study cited.

An interesting finding in this study was that even though aspartame-fed rats gained the same amount of weight as those not supplemented, they consumed significantly less feed, and hence calories. One might surmise that aspartame fed at this level makes rats more calorically efficient, so fewer calories are required to maintain the same body weight. This unfortunately seems to be counterproductive to a primary reason for consuming aspartame -- to lose weight by creating a calorie deficit. It is important to note that the effects of aspartame on feed intake were observed primarily in non-exercised rats. The exercised rats fed aspartame had feed intakes similar to those not exercised.

SUMMARY

The effects of aspartame and exercise on feed intake, weight gain, body composition, percent body fat and tissue lipid levels were studied in growing male Wistar rats. Comparisons were made within a 2 x 2 factorial design (n=9) where the main treatment effects were treadmill exercise (24 meters/min for 75 min/day, 5x/wk, 9 wks), and aspartame (0.6% in a nutritionally adequate purified diet).

Exercised rats gained less weight, had lower percent body fat, lighter fat pads (epididymal, retroperitoneal, and abdominal), less intramuscular fat (vastus lateralis, vastus medialis, and gastrocnemius lateralis), and lower serum cholesterol and triglyceride levels. Exercised rats also consumed less feed relative to body weight throughout the trial. Aspartame-treated rats had similar lipid contents in all tissues studied when compared to those not receiving aspartame. However, relative feed intake was significantly less in aspartame-fed rats.

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APPENDIX

APPENDIX TABLE 1
Request for aspartame from the Searle Company

REQUEST FOR ASPARTAME

Name Dr. Katharine K. Grunewald Date 11/25/84

Institution Dept. Foods and Nutrition, Justin Hall

Address Kansas State University

Manhattan, Kansas 66506

Phone Number (913) 532-5508 (office) or (913) 532-7010

Title or Function Assistant professor (nutrition)

Outline of Work to be Done:

The overall objective of the proposed study is to determine if aspartame supplemented-rats respond to exercise in a manner similar to those not fed aspartame. This objective will be accomplished by comparisons within a 2 x 2 factorial design (n=9) where the main treatment effects will be treadmill exercise (30 meters/min for 1 hr/day, 5x/wk), and aspartame (0.6% in an adequate AIN-76A, starch-modified diet). During the planned 8-week study the animals' voluntary feed intake and weight gain will be measured and feed efficiency will be calculated. At the end of the study the rats will be sacrificed and % body fat will be determined by hydrostatic weighing. Tissues affected by exercise will be weighed and composition will be determined: heart, lungs, liver, epididymal fat pads, kidneys, and 3 skeletal muscles from the hind legs. The muscles studied will include soleus (red, slow-twitch), extensor digitorum longus (white, fast-twitch), and anterior tibialis (mixed). We know that approximately 25 kg feed is needed to feed 18 young growing rats for 8 weeks and are requesting 150g aspartame to achieve a 0.6% aspartame diet.

Professional History

Degree B.S. (nutrition) University Univ. of Wis. Date 5/74

Degree M.S. (nutrition) University Univ. of Ky. Date 8/76

Degree Ph.D. (animal nutrition) University Univ. of Ky. Date 8/79

Relevant Publications: 1) Grunewald, K.K. and T.J. Tucker. 1984. Gastric emptying in exercised mice. Comp. Biochem. Physiol. In press. 2) Bulbulian, R., K.K. Grunewald and R.R. Haack. 1984. Effects of exercise duration on feed intake and body composition of Swiss albino mice. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. In press.

I agree that I will hold harmless G.D. Searle & Co., its subsidiaries, officers, directors and agents from any and all liabilities which may attach or flow from the above use of aspartame, including costs and attorneys' fees, if any.

Signature Katharine K. Grunewald Date 11/25/84

Katharine K. Grunewald

APPENDIX TABLE 2
Reply from the Searle Company

Searle Food Resources Inc.
Subsidiary of G.D. Searle & Co.
Box 1111
Skokie, Illinois 60076
Telex 28-420

March 21, 1985

Dr. Katharine K. Grunawald
Dapt. Foods and Nutrition, Justin Hell
Kansae State University
Manhettan, Kensas 66506

SEARLE

Dear Dr. Gruanwald:

Wa have ancloeed an edditional sample of esparteme par your requast. We egain ask thet you plaase:

e) Periodically inform ue of your prograee,

b) Provida to tha Offica of Scientific Affairs, NutraSwaat® Group, your dete for reviaw and commant prior to eubmission for publication.

If I can ba of eny further aseietance plaaea let me know.

Sincarely,

Daniel J. Skrypec, Ph.D.
Sciantific Affairs Analyst
NutraSweat® Canter

DS:nlc

Enclosura

APPENDIX TABLE 3
Density of water at different temperatures

density of water at different temperatures*

TEMPERATURE (°C)	D_w† (grams/ml.)
21	0.9980
22	0.9978
23	0.9975
24	0.9973
25	0.9971
26	0.9968
27	0.9965
28	0.9963
29	0.9960
30	0.9957
31	0.9954
32	0.9951
33	0.9947
34	0.9944
35	0.9941
36	0.9937
37	0.9934
38	0.9930
39	0.9926
40	0.9922

*Extracted from Weast, R. C. (Ed.): *Handbook of Chemistry and Physics*, 54th ed., Cleveland: The Chemical Rubber Company, 1967, p. F-11.

†Rounded to 0.0001.

APPENDIX TABLE 4
Determination of serum cholesterol (total, HDL, and LDL)

Isolab LDL-Direct Cholesterol Audit System
#QS-8160 (60 test)
Isolab Inc.
Innovative Biochemical Methodology
Drawer 4350
Akron, OH 44321 800-321-9632

Cholesterol Standards
Dow Diagnostics
The Dow Chemical Company
Indianapolis, IN 46268
200 mg/dl

I. Separation of alpha and beta fractions (HDL and LDL cholesterol)

1. Remove first the column's top cap, then the bottom closure. This order of opening is important - otherwise air will enter the column tip, interfering with free liquid flow.

2. Use the wide end of a Pasteur pipette to push the upper disc down until it contacts the top of the resin bed. Do not compress bed.

3. Allow the column to drain until the liquid level reaches the top disc, where flow will automatically stop.

4. Check to determine whether air may have entered the column during shipment. A few small air bubbles will not affect its performance. However, large volumes of air should be removed by tilting the top disc until the bubble escapes, then returning the disc to its original position.

5. Equlibrate the column bed by adding 1.0 ml of Alpha Fraction Elution Agent (Reagent #1) to the column. Allow column to drain. Discard eluate.

6. With the column positioned over a test tube (12 x 75mm, 5 ml), add 0.2 ml serum to the column, near or on the upper disc. Collect the eluate.

7. Add 1.0 ml of Alpha Fraction Elution Agent (Reagent #1) and collect the entire volume in the same test tube, for a total fraction volume of 1.2 ml. Mix well.

8. Place the column over a clean 12 x 75 mm tube.

9. Add 1.2 ml of Beta Fraction Agent (Reagent #2) and collect the entire volume. Mix well.
Fill column with saline or eluted Alpha Elution Reagent, recap and store for possible regeneration.

APPENDIX TABLE 4 (cont'd)

II. Quantitation of cholesterol (total, HDL, and LDL)

Incubator at 35 C

Cholesterol reagent should be reconstituted (50ml deionized distilled water/bottle) several hours or overnight before assay.

Disposable glass tubes 12 x 77 mm (5 ml culture tubes)

A. Dilution of standards and serum for total cholesterol (1:6 to match dilution of alpha and beta fractions during separation).

1. Pipette 0.2 ml of each STANDARD (200 mg/dl) or SERUM SAMPLE for total cholesterol into tubes.

2. Add 1.0 ml SALINE to each of the above tubes and vortex.

B. Cholesterol Determination

1. Pipette
   0.12 ml saline for blank (or distilled water)
   0.12 ml diluted serum for total cholesterol
   0.12 ml diluted standards
   0.12 ml alpha fraction
   0.12 ml beta fraction
   (run duplicates of each, except blank, but you may need more cholesterol reagent than is provided in the kit).

2. To each tube add 1.0 ml cholesterol REAGENT

   Cover with parafilm and invert several times to mix (gently)

3. Incubate all tubes at 37 C for 12-15 minutes.

4. Following incubation, add 1.0 ml SALINE to all tubes and vortex gently. (can add 2.0 ml saline to increase volume to 3 ml to read in Spec. 20).

5. Read BLANK - 100% transmittance, 0% absorbance as reference at 545 nm (directions indicate 500 $\pm$ 15 nm but Brinkmann probe has filter at 545nm). Read and record absorbance of STANDARD and SAMPLES.

   COMPLETE ALL READINGS WITHIN 30 MINUTES OF INCUBATION

APPENDIX TABLE 5
Computer program for statistical analyses

```
//NUTRASW  JOB (3916,01257,GFB4CE62,,7),'DIANNA',TIME=(1,59)
/*REGION       600K
// EXEC SAS
//SYSIN DD *
DATA ASPARTAM;
TITLE EFFECT OF EXERCISE AND ASPARTAME IN GROWING MALE RATS;
INPUT (ID EXERCISE ASPART MONWT1 SATWT1 MONWT2 SATWT2 MONWT5 SATWT5
MONWT4 SATWT4 MONWT5 SATWT5 MONWT6 SATWT6 MONWT7 SATWT7 MONWT8
SATWT8 MONWT9 SATWT9 MFFD1 SSFD1 MFFD2 SSFD2 MFFD5 SSFD5 MFFD4
SSFD4 MFFD5 SSFD5 MFFD6 SSFD6 MFFD7 SSFD7 MFFD8 SSFD8 MFFD9
BODYFAT LIVER KIDNEYS HEART ABDOMFAT EPIDFAT RETROFAT GASTROCL
GASTROCM VASTUSL VASTUSM)(3. 2*1. 18*3. 5*4.1 5.1 11*4.1 5.1 4.2
2*5.2 5*3.1 4*5.2);
TOTGAIN=SATWT9-MONWT1; DAYGAIN=TOTGAIN/61;
MFGAIN1=SATWT1-MONWT1; SSGAIN1=MONWT2-SATWT1;
MFGAIN2=SATWT2-MONWT2; SSGAIN2=MONWT3-SATWT2;
MFGAIN3=SATWT3-MONWT5; SSGAIN3=MONWT6-SATWT3;
MFGAIN4=SATWT4-MONWT4; SSGAIN4=MONWT5-SATWT4;
MFGAIN5=SATWT5-MONWT5; SSGAIN5=MONWT6-SATWT5;
MFGAIN6=SATWT6-MONWT6; SSGAIN6=MONWT7-SATWT6;
MFGAIN7=SATWT7-MONWT7; SSGAIN7=MONWT8-SATWT7;
MFGAIN8=SATWT8-MONWT8; SSGAIN8=MONWT9-SATWT8;
MFGAIN9=SATWT9-MONWT9;
AVMFGAIN=(MFGAIN1+MFGAIN2+MFGAIN5+MFGAIN4+MFGAIN5+MFGAIN6+
    MFGAIN7+MFGAIN8+MFGAIN9)/45;
AVSSGAIN=(SSGAIN1+SSGAIN2+SSGAIN5+SSGAIN4+SSGAIN5+SSGAIN6+
    SSGAIN7+SSGAIN8)/16;
TOTMFFD=MFFD1+MFFD2+MFFD5+MFFD4+MFFD5+MFFD6+MFFD7+MFFD8+MFFD9;
TOTSSFD=SSFD1+SSFD2+SSFD5+SSFD4+SSFD5+SSFD6+SSFD7+SSFD8;
AVMFFD=(TOTMFFD)/45; AVSSFD=(TOTSSFD)/16; TOTFD=TOTMFFD+TOTSSFD;
AVERFEED=(TOTFD)/61;
LIVERPER=(LIVER/SATWT9)*100; KIDPER=(KIDNEYS/SATWT9)*100;
HRTPER=(HEART/SATWT9)*100; GASTLPER=(GASTROCL/SATWT9)*100;
GASTMPER=(GASTROCM/SATWT9)*100; VASTLPER=(VASTUSL/SATWT9)*100;
VASTMPER=(VASTUSM/SATWT9)*100;
ABDOMPER=(ABDOMFAT/SATWT9)*100; EPIDPER=(EPIDFAT/SATWT9)*100;
RETROPER=(RETROFAT/SATWT9)*100;
DEPDFAT=ABDOMFAT+EPIDFAT+RETROFAT;
FDWTMF1=(MFFD1/5)/MONWT1; FDWTSS1=(SSFD1/2)/SATWT1; FDWTMF2=(MFFD2/5)/MONWT2;
FDWTSS2=(SSFD2/2)/SATWT2; FDWTMF3=(MFFD3/5)/MONWT3; FDWTSS3=(SSFD3/2)/SATWT3;
FDWTMF4=(MFFD4/5)/MONWT4; FDWTSS4=(SSFD4/2)/SATWT4; FDWTMF5=(MFFD5/5)/MONWT5;
FDWTSS5=(SSFD5/2)/SATWT5; FDWTMF6=(MFFD6/5)/MDNWT6; FDWTSS6=(SSFD6/2)/SATWT6;
FDWTMF7=(MFFD7/5)/MDNWT7; FDWTSS7=(SSFD7/2)/SATWT7; FDWTMF8=(MFFD8/5)/MONWT8;
FDWTSS8=(SSFD8/2)/SATWT8; FDWTMF9=(MFFD9/5)/MDNWT9;
MFWTFD=(FDWTMF1+FDWTMF2+FDWTMF5+FDWTMF4+FDWTMF5+FDWTMF6+FDWTMF7+
    FDWTMF8+FDWTMF9)/.09;
SSWTFD=(FDWTSS1+FDWTSS2+FDWTSS3+FDWTSS4+FDWTSS5+FDWTSS6+FDWTSS7+
    FDWTSS8)/.08;
AVWTFD=((MFWTFD*45)+(SSWTFD*12))/61;
IF EXERCISE=1 AND ASPART=1 THEN GROUP=1; IF EXERCISE=1 AND ASPART=2
    THEN GROUP=2; IF EXERCISE=2 AND ASPART=1 THEN GROUP=5; IF EXERCISE
    =2 AND ASPART=2 THEN GROUP=4;
CARDS;
$ADD DE_DATA
DATA SWEET;
INPUT (ID LIVFAT KIDFAT HRTFAT VMFAT VLFAT GMFAT GLFAT TG
    TOTCHOL LDL NDL GLU)(5. 4.2 6*5.2 5. 5*5.1 5.);
CARDS;
$ADD DATA_DE
DATA ALL; MERGE ASPARTAM SWEET; BY ID;
PROC SORT; BY GROUP; PROC PRINT; BY GROUP;
PROC MEANS; BY GROUP; VAR MONWT1--VASTUSM TOTGAIN DAYGAIN
    AVMFGAIN AVSSGAIN AVMFFD AVSSFD AVERFEED TOTFD LIVERPER KIDPER
    HRTPER GASTLPER GASTMPER VASTLPER VASTMPER DEPOFAT
    LIVFAT--GLU ABDOMPER EPIDPER RETROPER MFWTFD SSWTFD AVWTFD;
PROC ANOVA; CLASSES EXERCISE ASPART;
MODEL MONWT1 SATWT9 TOTGAIN DAYGAIN AVMFGAIN AVSSGAIN AVMFFD AVSSFD
    TOTFD AVERFEED BODYFAT LIVER LIVERPER KIDNEYS KIDPER HEART
    HRTPER GASTROCL GASTLPER GASTROCM GASTMPER VASTUSL VASTLPER
    VASTUSM VASTMPER DEPOFAT LIVFAT KIDFAT HRTFAT ABDOMPER EPIDPER
    RETROPER MFWTFD SSWTFD AVWTFD ABDOMFAT EPIDFAT RETROFAT
    VMFAT VLFAT GMFAT GLFAT TG TOTCHOL LDL NDL GLU=EXERCISE
    ASPART EXERCISE*ASPART;
/*
```

APPENDIX TABLE 6
Effects of aspartame and exercise on nine-week weight gains of growing male rats.

Measurement	-Exercise		+Exercise	
	-Aspartame	+Aspartame	-Aspartame	+Aspartame
			-g-	
Initial weight	71	70	68	57
	57	50	61	78
	66	72	59	66
	64	65	56	54
	58	59	95	90
	69	70	83	75
	67	83	92	65
	106	82	62	63
	61	78	73	75
	69±5*	70±4	72±5	69±4
Final weight	496	403	423	416
	421	442	428	424
	400	460	368	396
	412	500	387	476
	423	380	404	424
	437	430	418	385
	469	450	427	404
	475	510	415	414
	422	451	440	445
	439±11	447±14	412±8	420±9
Weight gain	425	333	355	359
	364	392	367	346
	334	388	309	330
	348	435	331	422
	365	321	309	334
	368	360	335	310
	402	367	335	339
	369	428	353	351
	361	373	367	370
	371±9	377±13	340±7	351±11

*Results are expressed as means ± SEM for 9 rats in each group.

APPENDIX TABLE 7
Effects of aspartame and exercise on average daily weight gains of growing male rats

Measurement	-Exercise		+Exercise	
	-Aspartame	+Aspartame	-Aspartame	+Aspartame
	-g-			
Average daily gain	-7.0	5.5	5.8	5.9
	6.0	6.4	6.0	5.7
	5.5	6.4	5.1	5.4
	5.7	7.1	5.4	6.9
	6.0	5.3	5.1	5.5
	6.0	5.9	5.5	5.1
	6.6	6.0	5.5	5.6
	6.0	7.0	5.8	5.8
	5.9	6.1	6.0	6.1
	6.1±.1*	6.2±.2	5.6±.1	5.8±.2
Average weekday (M-F) gain#	-7.0	7.9	5.3	5.4
	6.1	6.9	5.7	5.2
	5.1	6.2	4.8	4.7
	5.9	6.8	4.7	6.6
	5.9	3.7	4.9	4.8
	6.3	5.5	4.7	4.7
	6.6	5.6	5.1	4.8
	6.0	6.8	5.1	5.0
	5.9	6.2	5.6	5.3
	6.1±.2	6.2±.4	5.1±.1	5.2±.2
Average weekend (S-S) gain#	6.9	-1.5	7.2	7.2
	5.6	5.1	6.9	6.9
	6.5	6.9	5.8	7.5
	5.2	8.2	7.4	7.9
	6.3	9.6	5.4	7.3
	5.2	7.1	7.6	6.2
	6.6	7.1	6.6	7.8
	6.2	7.6	7.6	7.8
	6.0	6.0	7.2	8.1
	6.1±.2	6.2±1.0	6.9±.3	7.4±.2

*Results are expressed as means ± SEM for 9 rats in each group.
#Rats were exercised on weekdays (M-F) and rested on weekends (S-S).

APPENDIX TABLE 8
Effects of aspartame and exercise on average daily feed consumption of growing male rats

Measurement	-Exercise		+Exercise	
	-Aspartame	+Aspartame	-Aspartame	+Aspartame
	-g-			
Average daily feed consumption	24.5	19.0	18.6	19.3
	21.7	20.8	19.9	20.1
	20.3	20.5	18.6	18.6
	21.3	24.2	18.2	21.9
	20.4	18.9	19.4	20.5
	23.2	23.0	19.3	17.7
	22.7	22.5	21.7	19.4
	23.0	22.5	20.4	19.0
	21.9	20.9	21.1	21.1
	22.1±.5*	21.4±.6	19.7±.4	19.7±.4
Average daily feed consumption per 100g body weight#	9.8	8.6	8.1	9.0
	10.2	9.9	9.2	8.3
	9.5	8.9	9.8	8.7
	9.7	9.6	9.1	9.7
	9.8	9.0	8.1	8.5
	10.3	9.6	8.4	8.3
	10.4	9.0	9.2	9.0
	8.5	8.3	8.9	8.6
	10.0	8.4	8.6	8.6
	9.8±.2	9.0±.2	8.8±.2	8.7±.1

*Results are expressed as means ± SEM for 9 rats in each group.
#Relative feed intake calculated at end of each weekday or weekend period by dividing average daily feed consumption for that period by body weight for that period.

APPENDIX TABLE 9
Effects of aspartame and exercise on average weekday (M-F) feed consumption of growing male rats

	-Exercise		+Exercise	
	-Aspartame	+Aspartame	-Aspartame	+Aspartame
	-g-			
Average daily weekday feed consumption	24.1	18.5	17.4	18.2
	21.4	20.1	18.8	18.8
	19.9	19.6	17.4	17.5
	21.1	23.1	17.1	20.7
	20.0	17.8	18.6	19.2
	23.0	22.0	17.8	16.9
	22.1	21.4	20.5	18.3
	22.7	21.8	19.3	17.9
	21.8	20.2	20.0	19.8
	21.8±.5*	20.5±.6	18.5±.4	18.6±.4
Average daily weekday feed consumption per 100g body weight #	10.0	8.8	8.0	8.9
	10.5	10.1	9.1	8.1
	9.7	8.7	9.6	8.5
	10.0	9.5	8.9	9.6
	10.0	8.8	8.0	8.1
	10.6	9.4	7.8	8.2
	10.5	8.8	8.8	8.8
	8.5	8.2	8.8	8.4
	10.3	8.4	8.5	8.3
	10.0±.2	9.0±.2	8.6±.2	8.5±.2

*Results are expressed as means ± SEM for 9 rats in each group.
#Calculated on a weekly basis where average daily weekday feed consumption was expressed in terms of the initial Monday body weight.

APPENDIX TABLE 10
Effects of aspartame and exercise on average weekend (S-S) feed consumption of growing male rats

Measurement	-Exercise -Aspartame	-Exercise +Aspartame	+Exercise -Aspartame	+Exercise +Aspartame
		-g-		
Average daily weekend feed consumption	25.5	20.5	21.9	22.4
	22.5	22.9	23.1	23.5
	21.4	23.0	22.2	21.6
	21.7	27.3	21.4	25.2
	21.6	22.3	21.8	24.0
	24.0	25.8	23.5	20.1
	24.3	25.7	24.8	22.6
	23.9	24.8	23.5	22.1
	22.0	22.9	24.3	24.7
	23.0±.5*	23.9±.7	22.9±.4	22.9±.5
Average daily weekend feed consumption per 100g body weight#	9.3	8.3	8.6	9.4
	9.3	9.5	9.5	9.0
	9.1	9.3	10.6	9.1
	9.0	9.9	9.7	9.9
	9.1	9.7	8.5	9.5
	9.5	10.1	10.0	8.8
	10.2	9.8	10.2	9.7
	8.2	8.4	9.2	9.0
	9.1	8.5	8.8	9.3
	9.2±.2	9.3±.2	9.5±.2	9.3±.1

*Results are expressed as means ± SEM for 9 rats in each group.
#Calculated on a weekly basis where average daily weekend feed consumption was expressed in terms of the initial Saturday body weight.

APPENDIX TABLE 11
Effects of aspartame and exercise on percent body fat of growing male rats

Measurement	-Exercise		+Exercise	
	-Aspartame	+Aspartame	-Aspartame	+Aspartame
		-%		
Body fat	14.2	15.2	14.2	11.2
	17.5	19.5	13.2	11.2
	14.0	19.5	10.8	9.6
	18.0	22.6	17.1	14.0
	10.4	18.7	12.4	13.6
	17.5	19.5	17.1	14.4
	20.7	21.1	18.3	20.7
	19.1	19.5	15.2	13.6
	16.3	18.7	14.0	17.1
	16.4±1.0*	19.4±.7	14.7±.8	13.9±1.1

*Results are expressed as means ± SEM for 9 rats in each group.

APPENDIX TABLE 12
Effects of aspartame and exercise on epididymal fat of growing male rats

Measurement	-Exercise		+Exercise	
	-Aspartame	+Aspartame	-Aspartame	+Aspartame
	-g-			
Epididymal fat weight	6.4	7.0	4.5	5.5
	6.6	5.6	5.8	5.1
	3.6	8.0	4.7	4.1
	6.3	12.8	5.0	6.9
	5.1	5.2	4.2	6.9
	5.7	8.8	6.4	4.2
	10.7	9.1	7.8	6.4
	8.7	10.0	4.0	4.0
	6.6	8.5	5.9	7.2
	6.6±.7*	8.3±.8	5.4±.4	5.6±.4
Epididymal fat weight per 100g body weight	1.3	1.7	1.1	1.3
	1.6	1.3	1.4	1.2
	0.9	1.7	1.3	1.0
	1.5	2.6	1.3	1.4
	1.2	1.4	1.0	1.6
	1.3	2.0	1.5	1.1
	2.3	2.0	1.8	1.6
	1.8	2.0	1.0	0.9
	1.6	1.9	1.3	1.6
	1.5±.1	1.8±.1	1.3±.1	1.3±.1

*Results are expressed as means ± SEM for 9 rats in each group.

APPENDIX TABLE 13
Effects of aspartame and exercise on retroperitoneal fat of growing male rats

Measurement	-Exercise		+Exercise	
	-Aspartame	+Aspartame	-Aspartame	+Aspartame
	-g-			
Retroperitoneal fat weight - - -	8.8	10.0	3.3	4.8
	11.2	7.8	7.2	4.9
	5.3	12.7	7.0	3.6
	7.3	16.7	4.8	8.1
	5.4	6.3	3.3	7.4
	11.8	11.2	7.7	5.2
	14.4	12.4	7.8	7.0
	10.8	13.0	7.1	3.4
	6.9	12.0	6.7	7.3
	9.1±1.0*	11.3±1.0	6.1±.6	5.7±.6
Retroperitoneal fat weight per 100g body weight - - - - - -	1.8	2.5	0.8	1.2
	2.7	1.8	1.7	1.2
	1.3	2.8	1.9	0.9
	1.8	3.3	1.2	1.7
	1.3	1.7	0.8	1.7
	2.7	2.6	1.8	1.4
	3.1	2.8	1.8	1.7
	2.3	2.5	1.7	0.8
	1.6	2.7	1.5	1.6
	2.1±.2	2.5±.2	1.5±.1	1.4±.1

*Results are expressed as means ± SEM for 9 rats in each group.

APPENDIX TABLE 14
Effects of aspartame and exercise on abdominal fat of growing male rats

Measurement	-Exercise		+Exercise	
	-Aspartame	+Aspartame	-Aspartame	+Aspartame
	-g-			
Abdominal fat weight	7.5	6.4	3.1	4.6
	7.5	5.5	5.1	3.7
	4.8	8.1	3.1	3.3
	4.4	11.6	3.9	6.3
	4.5	4.2	2.5	5.0
	6.7	8.4	6.3	4.0
	10.9	8.0	5.9	6.1
	8.2	8.7	4.6	2.4
	6.2	6.8	5.1	5.5
	6.7±.7*	7.5±.7	4.4±.4	4.5±.4
Abdominal fat weight per 100g body weight	1.5	1.6	0.7	1.1
	1.8	1.2	1.2	0.9
	1.2	1.8	0.8	0.8
	1.1	2.3	1.0	1.3
	1.1	1.1	0.6	1.2
	1.5	2.0	1.5	1.0
	2.3	1.8	1.4	1.5
	1.7	1.7	1.1	0.6
	1.5	1.5	1.2	1.2
	1.5±.1	1.7±.1	1.1±.1	1.1±.1

*Results are expressed as means ± SEM for 9 rats in each group.

APPENDIX TABLE 15
Effects of aspartame and exercise on liver weight and lipid contents in growing male rats

Measurement	-Exercise		+Exercise	
	-Aspartame	+Aspartame	-Aspartame	+Aspartame
Liver weight (g)	16.08	11.70	12.24	12.97
	14.18	14.13	13.20	12.12
	13.42	14.89	9.70	12.31
	12.40	16.90	10.91	15.52
	11.64	10.39	12.52	13.42
	15.12	12.83	12.36	11.82
	14.74	13.24	12.80	12.16
	14.03	16.27	13.48	13.41
	11.85	14.34	12.52	12.64
	13.72±.51*	13.85±.69	12.19±.39	12.93 ±.38
Liver weight per 100g body weight (g)	3.24	2.90	2.89	3.12
	3.37	3.20	3.08	2.86
	3.36	3.24	2.64	3.11
	3.00	3.38	2.82	3.26
	2.75	2.73	3.10	3.17
	3.46	2.98	2.96	3.07
	3.14	2.94	3.00	3.01
	2.95	3.19	3.25	3.24
	2.81	3.18	2.85	2.84
	3.12±.08	3.08±.07	2.95±.06	3.07±.05
Liver lipid contents (%)	14.03	9.61	8.55	11.00
	9.32	8.87	9.16	9.00
	15.27	13.29	10.08	15.42
	9.61	9.16	8.40	6.26
	12.53	13.45	7.63	10.53
	8.87	13.90	11.61	12.53
	13.45	11.00	9.61	8.75
	11.90	10.24	15.87	8.75
	19.08	9.32	9.00	15.74
	12.67 ±1.10	10.98±.68	9.99±.83	10.89 ±1.06

*Results are expressed as means ± SEM for 9 rats in each group.

APPENDIX TABLE 16
Effects of aspartame and exercise on kidney weight and lipid content in growing male rats

Measurement	-Exercise		+Exercise	
	-Aspartame	+Aspartame	-Aspartame	+Aspartame
Kidney weight (g)- - - - - - - -	2.85	2.43	2.98	2.97
	2.47	2.89	2.92	3.02
	2.65	2.60	2.26	2.93
	2.40	3.08	2.40	3.54
	2.67	2.56	2.61	3.01
	2.46	2.81	3.02	2.76
	2.51	2.45	2.81	2.67
	2.89	2.90	2.69	2.88
	2.64	2.70	2.87	3.26
	2.62±.06*	2.71±.07	2.73±.09	3.00±.09
Kidney weight (both) per 100g body weight (g)- - - - - - - -	0.57	0.60	0.70	0.71
	0.59	0.65	0.68	0.71
	0.66	0.57	0.61	0.74
	0.58	0.62	0.62	0.74
	0.63	0.67	0.65	0.71
	0.56	0.65	0.72	0.72
	0.54	0.54	0.66	0.66
	0.61	0.57	0.65	0.70
	0.63	0.60	0.65	0.73
	0.60±.01	0.61±.01	0.66±.01	0.71±.01
Kidney lipid contents (%)- - - - -	7.51	7.63	6.90	6.68
	7.94	7.00	6.75	6.20
	6.43	6.83	6.64	6.52
	7.12	6.50	6.58	6.36
	7.24	7.00	6.62	6.18
	6.87	6.43	6.59	5.63
	6.10	6.46	6.32	6.39
	6.74	6.90	6.58	6.39
	6.32	5.31	6.11	#
	6.92±.20	6.67±.21	6.57±.08	6.29±.11

*Results are expressed as means ± SEM for 8-9 rats in each group.
#This sample was lost during analysis.

APPENDIX TABLE 17
Effects of aspartame and exercise on heart weight and lipid contents in growing male rats

Measurement	-Exercise		+Exercise	
	-Aspartame	+Aspartame	-Aspartame	+Aspartame
Heart weight (g)	1.40	1.29	1.35	1.32
	1.33	1.45	1.33	1.34
	1.22	1.29	1.19	1.23
	1.37	1.58	1.23	1.44
	1.21	1.19	1.24	1.33
	1.42	1.27	1.55	1.17
	1.41	1.16	1.18	1.36
	1.61	1.45	1.20	1.31
	1.24	1.38	1.45	1.43
	1.36±.04*	1.34±.04	1.30±.04	1.33±.03
Heart weight per 100g body weight (g)- - - - - - - - - -	0.28	0.32	0.32	0.32
	0.32	0.33	0.31	0.32
	0.31	0.28	0.32	0.31
	0.33	0.32	0.32	0.30
	0.29	0.31	0.31	0.31
	0.32	0.30	0.37	0.30
	0.30	0.26	0.28	0.34
	0.34	0.28	0.29	0.31
	0.29	0.31	0.33	0.32
	0.31±.01	0.30±.01	0.32±.01	0.32±.00
Heart lipid contents (%)- - - - -	4.58	4.07	4.23	4.51
	4.46	4.58	4.46	4.78
	4.51	4.07	4.62	5.03
	4.67	4.49	4.46	4.93
	4.93	4.99	4.23	4.46
	4.51	4.51	4.58	4.70
	4.55	4.73	4.65	4.22
	4.42	4.58	4.58	4.38
	4.08	4.65	4.74	#
	4.60±.06	4.52±.10	4.51±.06	4.63±.10

*Results are expressed as means ± SEM for 8-9 rats in each group
#This sample was lost during analysis.

APPENDIX TABLE 18
Effects of aspartame and exercise on vastus medialis weight and lipid contents in growing male rats

Measurement	-Exercise		+Exercise	
	-Aspartame	+Aspartame	-Aspartame	+Aspartame
Vastus medialis weight (g)- - - -	1.50	0.89	1.80	1.45
	1.24	1.19	1.30	1.48
	1.49	1.49	1.28	1.43
	1.21	1.18	1.21	1.41
	1.46	1.17	1.27	1.31
	1.31	1.27	1.18	1.40
	1.19	1.21	1.27	1.22
	1.34	1.43	1.42	1.32
	1.30	1.33	1.32	1.35
	1.34±.04*	1.24±.06	1.34±.06	1.37±.03
Vastus medialis weight per 100g body weight (g) - - - - - - -	0.30	0.22	0.42	0.35
	0.29	0.27	0.30	0.35
	0.37	0.32	0.35	0.36
	0.29	0.24	0.31	0.30
	0.35	0.31	0.31	0.31
	0.30	0.29	0.28	0.36
	0.25	0.27	0.29	0.30
	0.28	0.28	0.34	0.32
	0.31	0.29	0.30	0.30
	0.31±.01	0.28±.01	0.33±.01	0.33±.01
Vastus medialis lipid contents (%)- - - - - - - - - - - - - - -	3.05	2.56	2.04	2.53
	4.62	3.46	3.17	2.48
	2.92	2.77	3.04	2.88
	3.03	4.08	2.65	2.76
	2.67	2.94	2.33	2.45
	2.97	2.88	2.91	2.62
	3.27	3.22	2.88	2.44
	3.08	2.88	3.06	2.60
	2.82	3.62	2.60	2.71
	3.16±.19	3.16±.16	2.74±.12	2.61±.05

*Results are expressed as means ± SEM for 9 rats in each group.

APPENDIX TABLE 19
Effects of aspartame and exercise on vastus lateralis weight and lipid contents in growing male rats

Measurement	-Exercise		+Exercise	
	-Aspartame	+Aspartame	-Aspartame	+Aspartame
Vastus lateralis weight (g)- - - -	1.80	1.31	1.24	1.84
	1.32	1.55	1.46	1.66
	1.54	1.60	1.16	1.69
	1.61	1.54	1.48	1.82
	1.85	1.57	1.33	1.49
	1.59	1.50	1.54	1.69
	1.35	1.47	1.51	1.38
	1.45	1.66	1.58	1.75
	1.37	1.59	1.74	1.74
	1.54±.06*	1.53±.03	1.45±.06	1.67±.05
Vastus lateralis weight per 100g body weight (g) - - - - - - - - -	0.36	0.33	0.29	0.44
	0.31	0.35	0.34	0.39
	0.39	0.35	0.32	0.43
	0.39	0.31	0.38	0.38
	0.44	0.41	0.33	0.35
	0.36	0.35	0.37	0.44
	0.29	0.33	0.35	0.34
	0.31	0.33	0.38	0.42
	0.32	0.35	0.39	0.39
	0.35±.02	0.34±.01	0.35±.01	0.40±.01
Vastus lateralis lipid contents (%) - - - - - - - - - - - - - - - -	2.67	2.45	2.77	2.74
	3.99	2.81	2.67	1.93
	2.53	3.15	2.96	2.71
	3.56	3.72	2.48	3.02
	2.60	2.77	2.58	3.07
	2.59	2.44	2.53	2.44
	2.54	3.43	3.03	2.82
	2.84	3.17	2.46	2.36
	3.01	3.17	2.63	2.76
	2.93±.17	3.01±.14	2.68±.07	2.65±.12

*Results are expressed as means ± SEM for 9 rats in each group.

APPENDIX TABLE 20
Effects of aspartame and exercise on gastrocnemius medialis and lipid contents in growing male rats

Measurement	-Exercise		+Exercise	
	-Aspartame	+Aspartame	-Aspartame	+Aspartame
Gastrocnemius medialis weight (g)	1.32	0.95	1.02	1.22
	0.97	0.91	0.94	0.97
	1.10	1.40	0.88	1.11
	0.96	1.02	0.92	1.11
	1.24	1.02	0.93	1.10
	0.99	1.04	0.97	1.10
	0.95	0.98	1.04	1.01
	1.08	1.16	1.21	0.92
	1.08	1.13	1.07	1.06
	1.08±.04*	1.07±.05	1.00±.03	1.07±.03
Gastrocnemius medialis weight per 100g body weight	-0.27	0.24	0.24	0.29
	0.23	0.21	0.22	0.23
	0.28	0.30	0.24	0.28
	0.23	0.20	0.24	0.23
	0.29	0.27	0.24	0.26
	0.23	0.24	0.23	0.29
	0.20	0.22	0.24	0.25
	0.23	0.23	0.29	0.22
	0.26	0.25	0.24	0.24
	0.25±.01	0.24±.01	0.24±.01	0.25±.01
Gastrocnemius medialis lipid contents	3.12	2.65	2.69	2.63
	3.07	2.52	3.17	2.36
	2.92	2.13	2.86	2.48
	2.62	2.47	2.49	2.68
	2.77	4.27	3.00	2.50
	2.78	3.08	2.60	2.29
	3.38	3.27	3.52	2.95
	2.97	3.16	2.08	2.24
	2.12	2.43	2.35	2.38
	2.86±.12	2.89±.21	2.75±.15	2.50±.07

*Results are expressed as means ± SEM for 9 rats in each group

APPENDIX TABLE 21
Effects of aspartame and exercise on gastrocnemius lateralis and lipid contents in growing male rats

Measurement	-Exercise		+Exercise	
	-Aspartame	+Aspartame	-Aspartame	+Aspartame
Gastrocnemius lateralis weight (g) - - - - - - - - - - - - - - - -	2.14	1.47	1.51	1.85
	1.49	1.41	1.50	1.51
	1.77	1.70	1.23	1.71
	1.64	1.48	1.47	1.58
	1.87	1.49	1.72	1.62
	1.68	1.61	1.48	1.54
	1.46	1.55	1.61	1.47
	1.73	1.80	1.75	1.64
	1.75	1.54	1.55	1.65
	1.73±.07*	1.56±.04	1.54±.05	1.62±.04
Gastrocnemius lateralis weight per 100g body weight (g) - - - -	0.43	0.36	0.36	0.44
	0.35	0.32	0.35	0.36
	0.44	0.37	0.33	0.43
	0.40	0.30	0.38	0.33
	0.44	0.39	0.43	0.38
	0.38	0.37	0.35	0.40
	0.31	0.34	0.38	0.36
	0.36	0.35	0.42	0.39
	0.41	0.34	0.35	0.37
	0.39±.01	0.35±.01	0.37±.01	0.39±.01
Gastrocnemius lateralis lipid contents (g) - - - - - - - - - -	2.35	3.74	2.12	2.85
	3.54	3.25	3.05	2.43
	2.85	3.37	2.42	2.81
	2.51	3.56	2.80	2.61
	2.94	2.61	3.46	3.25
	3.00	3.41	3.25	2.97
	3.29	3.25	3.13	2.96
	3.31	2.93	2.88	2.23
	2.75	2.97	2.36	2.79
	2.95±.13	3.23±.12	2.83±.15	2.77±.10

*Results are expressed as means ± SEM for 9 rats in each group.

APPENDIX TABLE 22
Effects of aspartame and exercise on serum HDL, LDL, and TOTAL cholesterol in growing male rats

Measurement	-Exercise		+Exercise	
	-Aspartame	+Aspartame	-Aspartame	+Aspartame
	-mg/dl-			
HDL cholesterol	37.2	19.8	21.6	14.3
	24.8	40.9	35.4	24.4
	28.8	38.2	37.7	12.4
	24.7	----	37.0	29.0
	22.5	18.0	21.6	25.3
	26.1	23.7	13.9	24.9
	----†	18.7	24.1	12.5
	29.5	50.2	29.1	24.1
	21.2	42.3	23.7	26.1
	26.9±1.8*	31.5±4.5	27.1±2.7	21.4±2.2
LDL cholesterol	51.5	35.9	35.9	28.5
	52.0	51.0	47.4	30.3
	49.4	59.3	42.3	28.8
	41.2	----	41.2	32.2
	34.7	20.4	33.9	26.1
	45.3	33.5	26.5	27.4
	----	35.3	36.3	27.0
	41.5	44.0	39.0	30.3
	29.5	40.7	25.3	29.5
	43.1±2.9	40.0±4.2	36.4±2.4	28.9±0.6
Total cholesterol	79.5	50.1	44.1	63.0
	76.3	84.6	74.5	44.1
	65.8	76.8	72.2	41.2
	57.6	----	40.0	54.3
	55.5	32.2	58.4	49.8
	65.3	56.3	43.3	47.8
	----	50.6	60.6	42.7
	69.7	87.6	63.9	52.3
	50.6	80.5	49.8	54.4
	65.0±3.6	64.8±7.1	56.3±4.2	49.9±2.3

*Results are expressed as means ± SEM for 9 rats in each group.

†Could not draw blood from these rats.

APPENDIX TABLE 23
Effects of aspartame and exercise on serum triglycerides and glucose in growing male rats

Measurement	-Exercise		+Exercise	
	-Aspartame	+Aspartame	-Aspartame	+Aspartame
	-mg/dl-			
Triglycerides	98	121	55	174
	264	134	71	29
	105	113	49	61
	95	---	60	53
	143	43	79	148
	136	125	70	68
	---†	106	114	88
	179	221	153	60
	48	158	64	162
	133.5±23.2*	127.6±17.7	79.4±11.1	93.7±17.8
Glucose	144	68	108	163
	161	128	172	178
	182	189	184	166
	167	---	142	150
	136	135	106	148
	218	132	120	178
	---	132	162	295
	164	162	126	104
	112	148	178	136
	160.5±11.2	136.8±12.2	144.2±10.2	168.7±17.6

*Results are expressed as means ± SEM for 9 rats in each group.

†Could not draw blood from these rats.

THE EFFECTS OF ASPARTAME AND EXERCISE ON TISSUE LIPID LEVELS AND BODY COMPOSITION OF GROWING MALE RATS

by

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AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

The primary objective of this study was to determine if changes in tissue lipid levels which normally accompany exercise in rats are affected by aspartame feeding. Wistar male weanling rats were assigned to a 2 x 2 factorial design (n=9) where the main treatment effects were treadmill exercise (24 meters/min for 75 min/day, 5x/wk, 9 wks), and aspartame feeding (0.6% in a nutritionally adequate purified diet).

At the end of the 9-week trial, exercised rats gained less weight, had lower percent body fat, lighter fat pads (epididymal, retroperitoneal, and abdominal), less intramuscular fat (vastus lateralis, vastus medialis, and gastrocnemius lateralis), and lower serum cholesterol and triglyceride levels. Exercised rats also consumed less feed relative to body weight throughout the trial. Aspartame-treated rats had similar lipid contents in all tissues studied when compared to those not receiving aspartame. However, relative feed intake was significantly less in aspartame-fed rats.

Data suggest that aspartame feeding does not affect the changes in tissue lipid levels which accompany exercise in rats, but that feed intake relative to body weight is altered.