# Does essential fatty acid absorption change with aging?

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**Abstract** Linoleic acid, an essential fatty acid, is a prostaglandin precursor. We investigated the maximal capacity of the proximal jejunum and distal ileum to absorb linoleic acid in the unanesthetized rat. Groups of rats 1, 3, 12, and 28 months of age were studied. As the rats aged, their maximal capacity to absorb linoleic acid increased fivefold both in the jejunum and ileum. Since the intestinal wall content of linoleic acid remained relatively constant, age-related changes in mucosal surface area could not account for our observations. A decrease in the unstirred water layer thickness with aging was detected by measuring potential difference changes across the bowel. The total surface area of the unstirred water layer increased some fourfold and its resistance to linoleic acid transfer decreased fivefold with aging. These changes in the dimensions and characteristics of the unstirred water layer with aging may account for the fivefold increase in the maximal capacity of the small bowel to absorb linoleic acid.—**Hollander, D., V. D. Dadufalza, and E. G. Sletten.** Does essential fatty acid absorption change with aging? *J. Lipid Res.* 1984. **25:** 129–134.

**Supplementary key words** linoleic acid • prostaglandins • intestinal absorption

Linoleic acid is the major dietary essential fatty acid and a prostaglandin precursor in man (1). It is a polyunsaturated, 18 carbon, long chain fatty acid which cannot be synthesized endogenously in man but is readily produced by plants (2). Dietary deficiency of linoleic acid or its intestinal malabsorption causes impaired growth rate, skin disorders, increased platelet aggregation, abnormal blood clotting, decreased prostaglandin synthesis, and a wide variety of metabolic abnormalities (3). We have previously demonstrated that, in the unanesthetized rat, linoleic acid is absorbed by a concentration-dependent dual mechanism of transport. The absorption rate of linoleic acid is pH-dependent and is influenced by the concentration of bile acids, the simultaneous presence of other polyunsaturated fatty acids, and the resistance of the unstirred water layer (4, 5).

Since essential fatty acid deficiency could be a result of diminished intestinal absorption, we investigated the maximal intestinal absorptive capacity for linoleic acid in both young and old rats. We perfused the proximal and distal small bowel of unanesthetized rats separately, but simultaneously, with physiologic solutions containing linoleic acid, and measured its absorption rates by the two regions of the small bowel under a wide variety of experimental conditions. We also assessed the tissue accumulation of linoleic acid by the liver, jejunum, and ileum, and calculated the dimensions of the unstirred water layer by using linoleic acid as a probe molecule.

## MATERIALS AND METHODS

### Materials

[1-$^{14}$C]Linoleic acid (New England Nuclear, Boston, MA) with specific activity of 50.6 mCi/mmol was used as a tracer compound. The radiochemical purity of linoleic acid was >98% as checked on Silica Gel-G thin-layer plates developed in hexane–diethyl ether–acetic acid 70:30:1. Nonradioactive linoleic acid (Sigma Chemical Co., St. Louis, MO) was found by thin-layer chromatography to have less than 1% impurities. [$^{3}$H]Inulin (Amersham Searle Corp., Arlington Heights, IL) with specific activity of 0.9 Ci/mmol and radiochemical purity > 98% was used as a nonabsorbable marker to correct for water absorption or secretion (6). Recrystallized sodium taurocholate (Calbiochem Co., San Diego, CA) was found by thin-layer chromatography (7) to have less than 1% impurities. Analytical reagent grade sodium salts of monobasic or dibasic phosphate (J. T. Baker Chemical Co., Phillipsburg, NJ) were used as the components of the Krebs phosphate buffer (8). Sodium chloride, potassium chloride, magnesium sulfate heptahydrate, and dextrose were purchased from Mallinckrodt Scientific, Irvine, CA.

The intestinal perfusate consisted of a micellar solution

Abbreviation: UWL, unstirred water layer.

prepared by ultrasound radiation for 5 min at 70 watts of power with a sonicator (Artek Corp., Farmingdale, NY). The micellar solution contained 10 mM sodium taurocholate, 0.5 mM linoleic acid, tracer amounts of [$^{14}$C]linoleic acid and [$^{3}$H]inulin which were dissolved in a Krebs phosphate saline buffer at pH 6.5. The solution became optically clear after sonication and remained so for 24 hr at 20°C. The osmolarity of the final solution ranged from 286 to 304 mosmol/l.

The distribution of radioactive linoleic acid between its micellar and free monomeric forms was determined by passing the final perfusate through a UM-2 filter (Amicon Corp., Lexington, MA). Since less than 2% of the total radioactivity passed through the filter, we concluded that most of the linoleic acid was solubilized within the larger micellar particles which could not pass through the filter (4).

### Animals

Male Sprague-Dawley rats with known birthdates were purchased as weanlings from Charles River Laboratories, Wilmington, MA, and were raised in our animal colony. We followed the guidelines of the National Institute of Aging for the care and feeding of aging animals (9). The animals were fed standard rat chow which contained AIN-recommended vitamins and minerals and 20% by weight corn oil (approximately 40% linoleic acid). All of the animals were exposed to identical amounts of light and had identical opportunities for exercise. The animals were allowed food and water ad libitum and were not fasted prior to experimentation. Under our specific colony conditions, the animals' maximal life span was approximately 33 months.

### Experimental methods

Three to four animals of each age group were used for assessment of linoleic acid absorption. After the rat was anesthetized with ether, its jejunum and ileum were exposed and 12-cm proximal jejunal and 12-cm distal ileal segments were cannulated for perfusion. The perfusate solutions were infused through microbore tubing and the outflow from each intestinal segment was drained separately to the outside by an L-shaped glass cannula. The large (6 mm) internal diameter of the outflow glass cannula ensured that outflow from the perfused segments was not obstructed and that the intraluminal pressure remained relatively constant at, or below, atmospheric pressure. Both proximal and distal intestinal segments were flushed with saline to remove residual contents and then with air to remove residual wash solution. The abdominal cavity was then closed and the rat was placed in a plexiglass restraining cage which allowed minimal mobility of the rat but prevented dislodgement of the catheters (10). The rat was allowed to awaken and its body temperature was maintained at 37°C with a forced air heating device connected to a thermostatic temperature controller which was activated by a rectal temperature probe. Two syringe pumps were used to perfuse the two intestinal segments with a micellar perfusate solution at a constant flow rate of 0.5 ml/min.

The initial 20-min period of perfusion was discarded in order to allow the animals to reach a steady state of absorption, which was defined as a constant rate of disappearance of linoleic acid from the lumen per unit time. Each animal was then perfused for six 20-min periods with separate outflow collections. When the perfusion was completed, the intestinal segments were rinsed with saline at 0.5 ml/min for 10 min to remove residual perfusate. The rat was then killed with an overdose of ether and the perfused segments were removed and dried for 24 hr with a 5-g weight suspended from the dependent portion of each segment in order to achieve a standardized degree of stretching of the segments. After 48 hr of drying, the length and weight of the perfused segments were measured. The liver was removed and weighed after gentle blotting. A weighed aliquot of the liver was taken from a point in the mid-right lobe and kept frozen for future assessment of linoleic acid content.

### Radioactivity determinations

Duplicate 100-$\mu$l aliquots were taken from each 20-min perfusate collection and radioactivity was counted in a dioxane-based scintillation cocktail. Radioactivity was measured to a 1% counting error by using a liquid scintillation counter with automatic quench calibration at ambient temperature (Beckman LS 9000, Beckman Instruments, Irvine, CA).

### Calculations and statistical analysis

After the data had been corrected for water absorption or secretion (6, 10), the absorption rate of linoleic acid from each segment at each 20-min period was calculated separately. The disappearance rate of linoleic acid from the perfusate was calculated, and the value was expressed as absorption of linoleic acid per 100 cm of bowel per hr or per gram dry weight per hr. Accumulation of linoleic acid in the liver, jejunum, and ileum was measured by the total combustion of the tissues with a biological tissue oxidizer (Packard Instruments, Downers Grove, IL) which combusted the tissues and released tritiated $H_2O$ and $^{14}$C-labeled $CO_2$ separately (>98% recovery) enabling us to determine the liver and intestinal wall content of linoleic acid. Data were compared statistically with the Student's $t$ test (11).

### Determination of the unstirred water layer dimensions

Since linoleic acid has minimal aqueous solubility, its intestinal absorption may be limited by the unstirred water layer (UWL). The dimensions of the UWL in vivo were assessed in the following ways. The thickness of the UWL (d) was measured by a modification of the Diamond method, which involves the assessment of potential difference changes as the sodium chloride concentration in the perfusate is changed rapidly (12). Potassium chloride agar electrodes were placed in the peritoneal cavity and in the lumen of the small bowel. These electrodes were connected by potassium chloride bridges which, in turn, were connected to a volt meter and a strip chart recorder by standardized calomel electrodes. The intestinal lumen was then perfused alternately with solutions which were of equal osmolarity but which contained either 25 or 154 mM sodium chloride. Rapid manual changes of sodium chloride concentration induced potential difference changes (13). The rapidity of the change in potential difference is proportional to the thickness of the UWL which was calculated according to the following formula: $d = [(D)\ (t_{1/2})/0.38]^{1/2}$ where d is the thickness of the UWL, D is the diffusion coefficient of sodium chloride, $t_{1/2}$ is the half-time for the development of the new potential difference, and 0.38 is the correction factor originally derived by Diamond (12). The surface area of the UWL was calculated by the following formula (14, 15): $Sw = (Jd) \times (d)/(C_1) \times (D)$. In this formula Sw is the surface area of the UWL, Jd is the observed absorption rate of linoleic acid, d is the thickness of the UWL, $C_1$ is the concentration of linoleic acid in the intestinal perfusate, and D is the diffusion coefficient of linoleic acid. Once the surface area (Sw) of the UWL was determined, the resistance (R) of the UWL (15) was calculated by the formula: $R = (d)/(Sw) \times (D)$.

The free aqueous diffusion coefficient (D) of linoleic acid is $362.4 \times 10^{-6}$ cm$^2$/min as derived from the data of Westergaard and Dietschy (14). However, since linoleic acid is carried through the UWL within micellar particles and not in the free monomer, we used the value of $D = 120 \times 10^{-6}$ cm$^2$/min of the taurocholate micelles (16) for calculations of the Sw and R of the UWL.

TABLE 2. Linoleic acid absorption

| Age | Absorption | |
|---|---|---|
| | Jejunum | Ileum |
| *months* | *μmol/100 cm per hr* | |
| 1 | 7.7 ± 0.9 | 5.5 ± 0.9 |
| 3 | 22.6 ± 1.7[a] | 8.7 ± 1.0[a] |
| 12 | 27.7 ± 2.1 | 10.9 ± 1.2 |
| 28 | 40.0 ± 5.7[a] | 24.2 ± 3.6[a] |

Values are mean ± SEM. Number of animals at each age group as indicated in Table 1.

[a] Denotes significant ($P < 0.05$) difference from the value at the next lower age group.

## RESULTS

### Effect of aging on absorption rate

Three or four animals were studied at each age group with six different values obtained from each animal (**Table 1**). The mean ± SEM of linoleic acid absorption in each age group was calculated and tabulated (**Table 2**). The absorption rate of linoleic acid by the jejunal segments increased from 7.7 μmol/100 cm per hr at 1 month of age to 40.0 μmol/100 cm per hr at 28 months. The ileum's rate of absorption, which was lower than that of the jejunum, increased in a parallel fashion to that of the jejunum from 5.5 μmol/100 cm per hr at 1 month of age to 24.2 μmol/100 cm per hr at 28 months of age.

These measurements use the intestinal length as a common denominator for expression of the transport data. This method of comparing absorption rates between

TABLE 1. Characteristics of aging rats

| Age | Number of Rats | Body Weight | Liver Weight | Nominal Intestinal Surface Area |
|---|---|---|---|---|
| *months* | | *g* | *g* | *cm$^2$* |
| 1 | 4 | 119.7 ± 2.9 | 4.8 ± 0.3 | 108 ± 13 |
| 3 | 4 | 478.7 ± 22.4[a] | 16.4 ± 1.3[a] | 143 ± 10[a] |
| 12 | 4 | 677.2 ± 42.8[a] | 19.9 ± 1.2 | 163 ± 11 |
| 28 | 3 | 810.9 ± 41.9[a] | 22.4 ± 0.6 | 154 ± 12 |

Values are mean ± SEM. Intestinal surface area was derived from measurements detailed in reference 17.

[a] Denotes significant ($P < 0.05$) difference from the value at the next lower age group.

animals of varying ages is supported by our previous work (17) which clearly demonstrated a linear relationship between intestinal length and its surface area. In contrast, we did not find any correlation between the intestinal weight and its surface area as the rats aged (17). Nevertheless, since many investigators express absorption data per unit weight, we calculated our data per unit weight as well. Absorption of linoleic acid (μmol/g per hr) at 1, 3, 12, and 28 months by the jejunum was 7.5 ± 0.7, 12.3 ± 1.1, 12.8 ± 1.2, and 14.8 ± 2.1, and by the ileum 7.5 ± 0.9, 6.5 ± 0.4, 6.2 ± 0.6 and 9.5 ± 1.6, respectively.

The thickness of the UWL, when measured according to the potential difference change by our modification of the method of Diamond (13), decreased gradually from 318.5 to 268.8 μm as the animals aged from 1 to 28 months of age. Although the trend was in the direction of a decrease in thickness of the UWL, the difference did not reach statistical significance. As the animals aged, the surface area (14) of the UWL increased and the resistance (15) of the UWL decreased (**Table 3** and **Table 4**).

## DISCUSSION

We have previously delineated the intestinal absorptive mechanisms for linoleic acid in the rat. Using everted gut sacs in vitro and intestinal perfusions in vivo, we found that linoleic acid is absorbed by a concentration-dependent dual mechanism of transport. At low physiological concentrations (less than 1260 μM) absorption delineated saturation kinetics, while at high (2.5 to 4.2 mM) concentrations absorption took place predominantly by passive diffusion. We found that additions of metabolic inhibitors or decreases in the incubation temperature did not change the absorption rate of linoleic acid, indicating that the absorption mechanism was not energy-dependent (4, 5).

TABLE 3. Surface area of unstirred water layer

| | Surface Area of UWL (Jd)(d)/($C_1$)(D) | |
|---|---|---|
| Age | Jejunum | Ileum |
| *months* | *$cm^2$/100 cm length* | |
| 1 | 68.1 | 48.6 |
| 3 | 163.0[a] | 63.1[a] |
| 12 | 200.8 | 78.7 |
| 28 | 297.7[a] | 180.2[a] |

Values are means for each age of animals. The number of animals in each age group is indicated in Table 1.

[a] Denotes a significant ($P < 0.05$) difference from the value at the next lower age group.

TABLE 4. Resistance of unstirred water layer

| | R = (d)/(Sw)(D) | |
|---|---|---|
| Age | Jejunum | Ileum |
| *months* | *min/ml per 100 cm length* | |
| 1 | 3.89 | 5.45 |
| 3 | 1.33[a] | 3.46[a] |
| 12 | 1.08 | 2.75 |
| 28 | 0.75[a] | 1.24[a] |

Values are mean for each age group. Number of rats at each age is indicated in Table 1.

[a] Denotes significant ($P < 0.05$) difference from the next lower age.

In the present study we used a well delineated in vivo perfusion method (10) to study the effects of aging on the absorption of linoleic acid in the unanesthetized rat. We perfused the intestinal lumen of the proximal jejunum and distal ileum separately with physiological solutions containing linoleic acid in the physiological range of concentrations. The rats from our own animal colony were raised by us under optimal conditions for the study of aging (9). Under our specific set of environmental conditions, rats reach the upper limits of their life span by approximately 30 months of age. Rats used for this study (Table 1) developed normally and gained weight in the anticipated fashion (9). Examinations of the rats at the completion of experimentation showed them to be free of abnormalities.

The maximal absorptive capacity for linoleic acid in the jejunum increased from 7.7 at 1 month of age to 40 μmol/100 cm per hr at 28 months of age (Table 2). The maximal ileal capacity for linoleic acid absorption increased from 5.5 at 1 month of age to 24.2 μmol/100 cm/hr at 28 months of age. Thus, both the distal ileum and proximal jejunum, despite their known morphological and structural differences, demonstrated a similar increase, approximately fivefold, in maximal absorptive capacity for linoleic acid with aging (Table 2).

In previous experiments (17) we demonstrated that our animals do not undergo an increase in the nominal intestinal absorptive surface area as they age; therefore, our present observations cannot be the result of an increase in the anatomic surface area. When we measured the intestinal wall content of linoleic acid at the completion of each experiment, the amount of retained linoleic acid per 100 mg of tissue remained relatively stable both in the jejunum and ileum as the animals aged (**Table 5**). These findings argue against possible changes in the physical characteristics of the small intestinal mucosal surface or in small intestinal binding (18) of linoleic acid as the animals aged. Since the functional membrane surface area of the small intestine did not change with aging,

TABLE 5. Intestinal wall and liver content of linoleic acid

| Age | Jejunum | Ileum | Liver |
|---|---|---|---|
| *months* | *nmol/100 mg* | | *nmol/liver* |
| 1 | 488.6 ± 62.7 | 851.5 ± 28.4 | 61.1 ± 5.6 |
| 3 | 612.1 ± 58.4 | 846.9 ± 28.9 | 232.6 ± 24.7[a] |
| 12 | 544.9 ± 99.8 | 783.9 ± 81.7 | 242.0 ± 14.4 |
| 28 | 577.9 ± 12.4 | 797.2 ± 12.7 | 249.8 ± 15.6 |

Values are mean ± SEM. The number of animals at each age group is indicated in Table 1.

[a] Denotes significant ($P < 0.05$) change from the value at the next lower age group.

the ratio of the UWL surface area to membrane surface area increased with aging, providing an explanation for the present observations. Theoretically, it is also possible that the membrane permeability to linoleic acid or other lipophilic compounds could increase with aging. However, data to support this possibility are lacking at present.

The total liver content of linoleic acid remained constant from 3 to 28 months of age, suggesting that the rate of portal transport and removal of linoleic acid from the portal circulation does not increase with aging in this series of experiments (Table 5). Since the lymphatic circulation and not the portal is the major route of exit of long chain fatty acids (19), future studies of possible changes in lymphatic appearance rate of linoleic acid with aging will be carried out.

The unstirred water layer (UWL) dimensions and resistance form a major rate-limiting step in regulating the uptake of fatty acids by the absorptive cells (20). The UWL can be characterized by at least three different approaches. The thickness of the UWL can be determined by assessing potential difference changes across the small bowel following rapid alterations in the intraluminal sodium concentration (13). We found a gradual decrease in the thickness of the unstirred water layer (from 318.5 to 268.8 micrometers) as the animals aged. On the other hand, when the surface area of the UWL was assessed using linoleic acid as a probe (14), a fourfold increase in the surface area of the UWL was found both in the jejunum and the ileum as the animals aged (Table 3). The fourfold increase in the surface area of the UWL would increase the absorption rate of linoleic acid even if the resistance and thickness of UWL remained constant (14, 15, 20). The increase in the UWL surface area would allow a greater amount of linoleic acid to diffuse across this barrier per unit time. The increase in the surface area of the UWL could be secondary to an increase in the proportion of the villus depth which is used for absorption of linoleic acid as animals age. If absorption occurs further down the villus due to possible widening of the intervillus spaces or due to different rigidity characteristics of the villi themselves, the unstirred water layer would tend to interdigitate more deeply between villi rather than remain as a flat sheet covering the tips of the villi only. These possible changes would result in an increase in the surface area (14, 15, 20) of the UWL (Table 3) and promote an increase in linoleic acid absorption. When the resistance (15) of the UWL was calculated (Table 4), a significant fall in the resistance was observed both in the jejunum and the ileum as the animals aged. Thus, the increase in the absorptive capacity of the small bowel for linoleic acid as the animals aged is partly due to decreased resistance for linoleic acid transfer across the UWL. We do not have data to explain the observed decrease in UWL resistance (Table 4). It could be associated with changes in the viscosity of the UWL itself secondary to changes in the mucus composition or concentration in the UWL (21).

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