Absorption of iron from gut into blood: sex- and time-related studies in rats[1]

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Summary. As judged from 2-h blood level curves, adult female rats absorbed more Fe^{II} per cm^2 of gross duodenal mucosa than adult male rats. By contrast, the 2-h blood level curves per cm^2 of mucosa of proximal jejunum did not differ significantly in male and female rats although in both sexes, iron was absorbed more efficiently from the duodenum.

It has long been known that the duodenal mucosa of the rat normally absorbs inoganic iron more efficiently than do other segments of intestinal mucosa[2], but sex- and time-related comparisons of blood levels after absorption of iron from loops of rat duodenum and jejunum have not been published before. We determined blood levels of tracer ^{59}Fe at intervals after injection of various doses of ^{56}Fe together with the tracer into closed loops of either duodenum or jejunum. Although mature male and female rats absorbed iron from the proximal jejunum with equal efficiency, absorption from the duodenum was much better in females. This phenomenon may account for the significantly higher iron stores in the livers, spleens and kidneys of female rats[3-6].

Materials and methods. Sets of male and female Sprague-Dawley rats weighing either between ~ 170 and 250 g or between 300 and 400 g were used. The differences in weight ranges were either due to sex (M > F at equal age) or to unavoidable logistic problems, and they were taken into account in the statistical analysis (see below). In each experimental series the spread of weights was within 20 g. These rats had been fed Purina chow and given distilled water ad libitum. As a rule, the animals were fasted (water ad libitum) for 20 h, in 1 experiment for 5 h. Under pentobarbital anesthesia, supplemented by diethyl ether, the abdomen was opened by a midline incision. The duodenum was isolated by means of cotton tape ligatures, 1 ligature being placed just beyond the pylorus, the other just above the duodeno-jejunal junction. In other rats, the first ~ 5-6 cm of jejunum were isolated in similar fashion with cotton tape ligatures, taking care to avoid injury to mesenteric blood vessels. Then, one of the tail veins was incised to draw 2 samples of blood into 20-µl capillary tubes. Prompt hemostasis was produced by pressure with a cotton sponge. Immediately thereafter, 0.4–0.5 ml of an aqueous solution containing inorganic iron and other electrolytes, with ascorbic acid and mannose, was injected into the lumen of the isolated loop of gut from a syringe with a 1.25 cm No. 27 needle, taking care to avoid leakage. The solution, similar to that used by Halliday et al.[7], contained the following ingredients: NaCl: 105 mM; KCl: 5 mM; $CaCl_2$: 0.1 mM; Mannose: 40 mM; Tris buffer (pH 6.5): 4 mM; ascorbic acid: 8 mmoles for each mmole of added ^{56}Fe; $^{59}FeCl_3$ in 0.1 M HCl: as required to produce either 3 or 6 µCi in 0.4–0.5-ml; $^{56}FeSO_4 \cdot 7\ H_2O$ (non-radioactive): as required. $^{59}FeCl_3$ (New England Nuclear, Boston, Mass.) had specific activities between 25 and 30 mCi/mg (30 mCi/ml).

After injection of the test solution into the intestinal lumen, gauze sponges, soaked in warm 0.9% NaCl solution, were spread over the exposed loops, the abdominal wall was placed over them, and covered in turn by wet gauze to avoid drying of serosal surfaces. The rats were kept under anesthesia until the end of each experiment, generally for over 120 min. After 5, 10, 30, 60, and 120 min, duplicate samples of 20 µl blood were drawn into 20-µl capillary tubes. After 120 min, the ligated segment of intestine was excised and opened longitudinally to inspect its contents and the mucosa. Little fluid was present in the lumen at this time. In a few instances slight bleeding had occurred from one or the other site of ligation. The opened segment was spread flat on a piece of absorbent paper and its length and

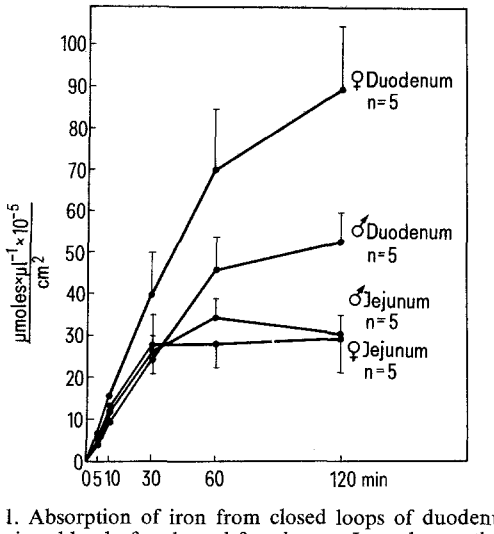

Figure 1. Absorption of iron from closed loops of duodenum or jejenum into blood of male and female rats. In each case the dose was 0.04 µmoles ^{56}Fe, with 3 µCi of ^{59}Fe as tracer. n, number of rats in each group; bars, SEM. Combination of probabilities in males and females at each time point for absorption from duodenum gives p = 0.01. Differences between absorption from duodenum and jejunum are significant at 120 min in females (0.025 > p > 0.01) and males (0.025 > p > 0.01).

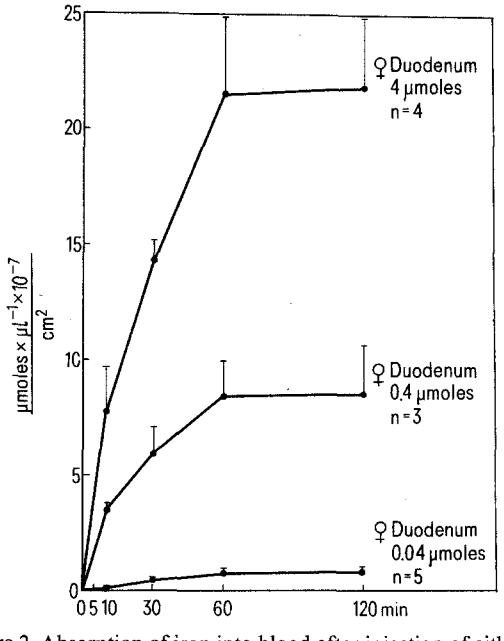

Figure 2. Absorption of iron into blood after injection of either 0.04 or 0.4 or 4 µmoles of ^{56}Fe, with 6 µCi ^{59}Fe as tracer, into closed loops of duodenum. n. number of rats; bars, SEM.

width (circumference) measured to determine the gross area of mucosa.

The blood samples in capillary tubes were counted in a Beckman gamma counting spectrometer. After subtraction of background, results were calculated as counts per 20 µl blood per cm^2 of mucosa. Knowing counting efficiency, dpm/µCi of ^{59}Fe, and µCi^{59}Fe/µmole of ^{56}Fe in the solution injected into the isolated segment of gut, the quantity of iron represented by the radioactivity in the samples of blood was estimated and expressed as $\mu moles \times \mu l^{-1} \times 10^{-5}$ per cm^2 of mucosa. This assumes that specific activity of the isotope remained unaltered during the absorption process. We also assumed that in each of the 2 stated weight ranges of rats, variation in blood volume would affect results insignificantly. Linear regression of absorption of Fe per cm^2 mucosa was tested as a function of body weight. As shown in the table, 95% confidence intervals of the correlation coefficients were so broad as to be insignificant in both female and male rats. Duplicate counts of blood samples were within 2% of each other, as a rule. A t-test was used to evaluate the significance of differences between blood levels of ^{59}Fe after absorption from duodenum or jejunum, in males and females. The significance of differences between absorption curves was also tested by combining probabilities of data at each time point and applying a χ^2-test according to Fisher[8].

Results. In male and female rats fasted for 20 h, absorption of a dose of 0.04 µmoles iron was greater per cm^2 of duodenum than it was per cm^2 of jejunum (fig. 1). Also, duodenal absorption was more efficient in female rats than in males (fig. 1). Thus, blood levels rose more rapidly in females during the first h and reached higher levels in 2 h. With respect to absorption from jejunum, there was no significant difference between male and female rats (fig. 1). As judged from blood levels, the rates of absorption of a dose of 0.04 µmoles from duodenum and jejunum during the first 30 min were almost the same in males, but in females the duodenal rate tended to be higher during the initial 30 min. Thereafter, the rates of absorption from the jejunum were always lower than the duodenal rates, in both females and males. With absorption from jejunum, blood levels of ^{59}Fe remained approximately the same after 30–60 min had elapsed, indicating that clearance from the blood and rate of absorption had reached equilibrium. With absorption of 0.04 µmoles from duodenum, blood levels of ^{59}Fe rose markedly until 60 min had elapsed, and then tended to level off. Similar results, but with considerably higher blood levels, were obtained with doses of 0.4 and 4 µmoles, respectively (fig. 2). These data indicate that, within the dose range of 0.04–4 µmoles, blood levels are compatible with first order absorption kinetics. When the dose of iron was increased to 40 µmoles, high blood levels of iron were reached in 10 min (fig. 3). After injection of 40 µmoles into duodenum, the blood level reached a peak about 30 min later and thereafter dropped rapidly. When 40 µmoles were injected into jejunum, the peak blood level was reached about 10 min later and collapsed subsequently (fig. 3). The blood level curves in figure 3 indicate rapid cessation of absorption after the peak, according to well-known kinetic principles[9]. This collapse of absorption suggests severe damage to the absorbing mechanism in the gut, presumably caused by excess iron.

Discussion. Intrinsic properties of the duodenal mucosal cells may account for its relative efficiency in absorbing inorganic iron. Absence of biliary ascorbate from the ligated jejunum cannot account for the lower absorption from the jejunum, as Conrad and Schade[10] have suggested in another context, since iron was given with ascorbate (e.g. 0.04 µmoles Fe with 3.2 µmoles of ascorbate). It is not known whether rat duodenum has more iron-absorbing cells per unit area than rat jejunum, but even with the same numerical density of iron-absorbing cells there could be differences related to absorbing cell surface areas, or to number of 'receptors' for iron (whatever their nature may be), or to rate of transport of iron across the mucosal bar-

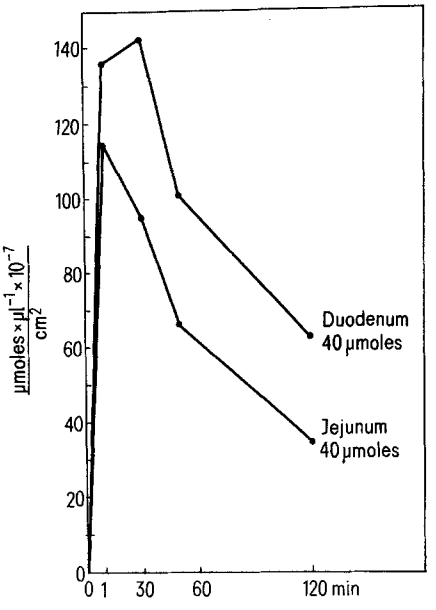

Figure 3. Absorption of iron into blood after injection of 40 µmoles of ^{56}Fe, with 24 µCi ^{59}Fe, into duodenum of female rats. Data from individual rats.

Correlation coefficients and 95% confidence intervals for linear regression of absorption per cm^2 mucosa on body weights

Sex	Absorption time (min)*	Duodenum n	r	I	Jejunum n	r	I
Male	5	9	−0.56	−0.87, +0.17	8	+0.38	−0.42, +0.83
	10	9	−0.58	−0.88, +0.15	9	+0.19	−0.52, +0.73
	30	8	−0.27	−0.80, +0.50	9	−0.02	−0.65, +0.63
	60	9	−0.58	−0.88, +0.15	9	−0.44	−0.83, +0.32
	120	9	−0.53	−0.86, +0.22	8	−0.59	−0.90, +0.19
Female	5	5	−0.68	−0.96, +0.42	5	+0.23	−0.73, +0.87
	10	5	−0.41	−0.90, +0.65	5	+0.45	−0.62, +0.92
	30	6	−0.44	−0.88, +0.52	6	+0.64	−0.32, +0.94
	60	5	−0.28	−0.88, +0.72	5	+0.83	−0.13, +0.97
	120	5	−0.73	−0.96, +0.35	5	+0.94	+0.28, +0.98

*Dose: 0.04 µmoles Fe. n, number of rats; r, coefficient of correlation for the absorption ($\mu moles \times \mu l^{-1} \times 10^{-5}/cm^2$). I, 95% confidence interval Dunn[13], p. 175.

rier. Of various possiblities, variation in density of surface receptors for iron has not been investigated. Our results are compatible with the findings of Wheby et al.[1], who determined mucosal 'uptake' and 'transfer' of iron from closed loops of rat duodenum and proximal jejunum by counting radioactivity of ^{59}Fe in gut and carcass.

Normally, there is more storage iron in liver, spleen and kidneys in mature female rats than is contained in these organs in mature male rats[3–6]. Linder et al.[5] have provided evidence indicating that the greater accumulation of iron in mature female rats is related to a higher level of ferritin synthesis, at least in the liver. Since the level of intracellular ferritin reflects cellular uptake of iron[11], Linder et al.[5] suggested that female rats absorb iron more efficiently from the gut than do male rats. Long ago, Otis and Smith[12] noted better absorption of dietary iron by female rats. Our findings suggest that this difference could be due to more efficient absorption of dietary iron from the duodenum in females. As shown in figure 1, the blood iron curves after absorption of iron from the duodenum differed significantly in male and female rats, yet there was no significant sex difference in the curves when iron was absorbed from the proximal jejunum. The reason for the sex difference in absorption from the duodenum is unknown, but cannot be ascribed to iron deficiency in females with ample stores of iron.

The blood iron curves shown in Figures 1 and 2 indicate that absorption was continuous during the 2-h interval and that egress of iron from the blood never exceeded ingress. The curves are compatible with first order absorption kinetics or perhaps with Michaelis-Menten kinetics (mixed first and zero order). However, the highest dose given (40 µmoles) could have damaged the absorbing cells in the gut since blood iron levels diminished precipitously after a rapid initial rise and no plateau level developed.

The possibility that there are differences in surface densitiy of receptors for inorganic iron along the course of the small intestine deserves detailed investigation.

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Cold-induced changes in fatty acid composition of rat brown fat during the perinatal period

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Summary. Cold exposure of the newborn rat has little effect on the fatty acid composition of triglycerides and phospholipids up to the 14th day. During the 3rd week, cold exposure inhibits the involution of brown fat observed in the warm-exposed rat.

Brown adipose tissue (BAT) is known to be a site of non-shivering heat production in newborn rats as well as cold-adapted adult animals. Changes in fatty acid composition occur during the development of BAT[1,2] and after several weeks of cold exposure of the rat[3,4]. Furthermore, it has been observed that the increase in BAT weight is larger in newborn rats exposed to cold than in animals kept at the mother's thermal neutrality, despite a drop in the lipid content of the former[5]. In the present paper an attempt is made to analyze the fatty acid composition of the triglycerides and phospholipids of BAT from foetuses and newborn rats in relation to development and ambient temperature.

Material and methods. Female Sprague-Dawley rats were placed at either 16 °C or 28 °C on the 15th day of gestation. At birth, the newborns were kept with their mother at these ambient temperatures. 20-day-old foetuses and 1-, 3-, 7-, 14- and 21-day-old rats were killed by decapitation; interscapular brown adipose tissue (BAT) was rapidly removed, weighed and immersed in liquid nitrogen.

Total lipids of BAT and of milk from the stomach of 2-day-old littermates were extracted according to procedure of Folch et al.[6]. Triglycerides (TG) and phospholipids (PL) were separated by TLC on silica gel. Phospholipid phosphorus was determined using Bartlett's method[7]. TG weight was obtained from the difference between the total lipid weight and the PL weight. The relative amounts of the various TG or PL fatty acids were estimated using GLC.

Results and discussion. Triglycerides. The total lipid content of BAT was low in the foetuses (4.7%) (fig. 1) and about 70% of these lipids were TG. Their fatty acid composition (fig. 2) was characterized by a predominance of saturated fatty acids (more than 50% palmitic acid). No difference was observed in relation to the mother's ambient temperature.

From birth to the 3rd day post-partum, after the onset of suckling, the total lipid content of BAT increased more rapidly in 28 °C-exposed than in 16 °C-exposed rats (fig. 1). The fatty acid composition was greatly changed (fig. 2); there was a decrease in palmitic acid and an increase in oleic and linoleic acids. On day 3, in both groups, the fatty acid composition of brown fat TG was very similar to that of the milk (about 8% lauric and myristic acids; 5% palmi-