Influence of Pregnancy and/or Exercise on Intestinal Transport of Amino Acids in Rats (35003)

M. C. DUGAS, R. L. HAZELWOOD, AND A. L. LAWRENCE
Department of Biology, University of Houston, Houston, Texas 77004

Little experimental data have been reported relative to the effects of pregnancy on dynamic aspects of intestinal transport of nutritive substrates. Enlargement of the intestine is known to occur during the 21-day gestation period of the rat (1); furthermore, *in vivo* studies document the fact that transport of *d*-glucose is augmented during days 12–15 of pregnancy in this species (2, 3). Of the available amino acids, only methionine and glycine have been used to study the effect of pregnancy on amino acid transport. Pregnancy had no effect on *in vivo* transport of ^{35}S-*dl*-methionine as observed on days 10, 16, and 21 of pregnancy (4). Also *in vivo* studies by Craft (5) demonstrated no alteration in glycine transport at 18–21 days of pregnancy whereas other workers using an *in vitro* sac method reported increased glycine absorption during pregnancy (3).

The purpose of the present *in vitro* study was to compare the effects of three different gestational periods on gut transport of several amino acids. Also, it was intended that information would be gained as to the influence of exercise on amino acid translocation in rats as well as the effect of exercise superimposed on pregnancy on this intestinal function.

Methods. All rats used were adult female Holtzman rats, weighing 190–210 g at the start of the experimental period, fed Purina Lab Chow and water *ad libitum*, and housed at a temperature of 21 ± 1°. Daily vaginal smears were taken and the presence of sperm was considered day 0 of the gestation period. Exercised groups were run 30 min/day at 15 m/min on a Collins Rodent Treadmill, Model 297. Pilot studies in which rats ran at a pace of 7, 15, 20, 25, or 30 m/min were conducted and from the body weight change, adrenal weight, and gut transport of lysin data, 15 m/min was chosen as the most desi able treadmill velocity. Groups consisted c rats pregnant for 7, 14, or 21 days; ra exercised seven consecutive days from 0– 7–13, and 14–20 days of pregnancy; and on group exercised 0–20 days of pregnancy these groups are referred to as 7-P, 14-I 21-P, 0-6PE, 7-13PE, 14-20PE, and 0-20PI respectively.

Rats were sacrificed by cervical dislocatio 24-hr after the last exercise period, the sma intestine removed, measured and placed int Krebs–Ringer buffer solution. A 25-cm seg ment was taken 21–46 cm proximal to th ileocecal junction, everted over a glass ro and divided into three equal lengths. The *i vitro* method used was identical to that previ ously reported (6) with the following excep tions: a 30-min incubation period was em ployed; the Krebs–Ringer buffer initiall contained 1 μmole of an l-amino acid an 0.0024 μCi ^{14}C activity per ml; and no sugar For counting, 100 or 200 λ of the serosa fluid was added to 15 ml of dioxane cocktai (100 g napthalene, 6 g 2,5-diphenyloxazole 100 ml H_2O, and 900 ml dioxane per liter o cocktail) and counted in a liquid-scintillatio spectrometer for estimation of the fina amount of ^{14}C-labeled amino acid. L-Lysine 1-^{14}C, l-proline-5-^{14}C, and l-alanine-1-^{14}C were used. Since significant net water move ment was not observed in any amino aci series, calculations were based on initial flui volumes. Because of regional gut difference in the rate which amino acids are transporte it appeared more meaningful to report trans port data here as net accumulation in serosa fluid as μmoles/g dry gut weight after a 30-min incubation period at 37°. Descendin paper chromatography was carried out on th

TABLE I. Effect of Pregnancy and/or Exercise on Body Weight, Adrenal Weight, Gut Length, and Conceptus of Rats.

Group	No. of observ.	Body wt change (g)	Adrenal gland mg	Adrenal gland mg/100 g body wt	Gut length cm	Gut length cm/100 g body wt	Conceptus Total wt (g)	Fetuses Av wt (g)	Placenta Av wt (g)
Virgin controls									
7-day	10	+ 12.2	60.5 ± 3.1	28.4 ± 1.5			—	—	—
14-day	7	+ 26.3	64.6 ± 4.4	28.8 ± 0.8	113 ± 1.2	49.7 ± 0.9	—	—	—
21-day	19	+ 25.7	60.7 ± 1.9	26.5 ± 0.8			—	—	—
Exercised virgins									
0– 7 day	15	+ 5.9	60.2 ± 1.6	27.4 ± 0.7	114 ± 1.2	52.8 ± 0.6	—	—	—
0–21 day	10	+ 23.0	62.2 ± 1.4	27.2 ± 0.8	110 ± 1.0	48.0 ± 0.9	—	—	—
Pregnant rats									
7-day	16	+ 21.9	59.2 ± 1.2	26.6 ± 0.6	114 ± 1.5	51.0 ± 0.8	0.56 ± 0.1	—	—
14-day	15	+ 53.6	65.5 ± 1.7	26.0 ± 0.7	113 ± 2.4	45.1 ± 0.9	10.3 ± 0.8	0.26 ± 0.03	0.22 ± 0.01
21-day	14	+125.4	68.9 ± 2.0	21.4 ± 0.7	122 ± 1.8	36.4 ± 0.8	73.9 ± 3.1	4.57 ± 0.15	0.73 ± 0.03
Exer. + pregnancy									
0– 6 day	3	+ 22.3	54.9 ± 4.5	25.1 ± 2.0	113 ± 2.9	51.5 ± 0.8	0.53 ± 0.1	—	—
7–13 day	3	+ 40.3	72.7 ± 3.0	31.4 ± 0.4	118 ± 2.9	52.2 ± 2.5	8.1 ± 0.8	0.15 ± 0.01	0.16 ± 0.01
14–20 day	10	+108.4	65.7 ± 1.4	21.4 ± 0.6	120 ± 2.2	38.9 ± 0.8	67.8 ± 3.0	4.54 ± 0.88	0.62 ± 0.04
0–20 day	3	+121.3	66.9 ± 0.6	21.0 ± 0.4	118 ± 1.7	37.1 ± 0.7	76.7 ± 3.7	4.88 ± 0.02	0.58 ± 0.01

final serosal and mucosal samples in a butanol:acetic acid:water system (4:1:2), followed by autoradiography. One spot was observed indicating a lack of metabolism of the test amino acids.

Results. The physiological impact of treadmill exercise, pregnancy, or both exercise and pregnancy on the adult female rat is presented in Table I. The effects of pregnancy on body weight increase, absolute adrenal weight, and gut elongation are obvious during different periods of gestation. However, when 7 days of running was performed during the three different periods of gestation there was a tendency for decreases to occur in body weight gain, size of conceptus, early fetal weights, and in mean placental size. Gut length was not affected by the concomitant physiological stresses of exercise and pregnancy, the absolute length being approximately the same as that in pregnant nonexercised rats ($p > .05$). It is of interest to note that when pregnant rats were exercised 21 consecutive days (0-20PE) virtually complete homeostatic compensation took place as judged by all observed parameters, a possible exception being that of placental development (21-P *vs* 0-20PE).

The effect of pregnancy and/or exercise on net serosal accumulation of amino acids by the rat gut are presented in Table II. After 7 days of exercise, there was a decrease in the net serosal accumulation of all three amino acids though only the value for lysine is significantly different ($p < .05$) from the virgin controls. The accumulation of alanine was significantly less ($p < .01$) after 21 days of exercise than that of the 7-day exercised or nonexercised virgin rats. At the end of the first week of pregnancy there was a significant increase ($p < .01$) in the net serosal accumulation of lysine but no significant changes occurred with alanine or proline. No significant changes in the net serosal accumulation of any of the three amino acids was seen during the terminal 2 weeks of pregnancy. Exercise had no significant effect on the net serosal accumulation of alanine in the 0-6PE and 7-13PE groups. Contrarily, significant decreases occurred in the net accumulation of alanine ($p < .01$) and lysine ($p < .01$) in the 14-20PE groups. Again, exercise of pregnant

TABLE II. Effect of Pregnancy and/or Exercise on Net Serosal Accumulation of Amino Acids by the Rat Gut (μmole/g dry gut weight).

Group	Alanine	Lysine	Proline
Virgin controls	5.89 ± 0.6[a] (12)	2.63 ± 0.3 (24)	3.37 ± 0.7 (12)
Exercised virgins			
0– 7 day	5.15 ± 0.6 (26)	1.63 ± 0.3[b] (11)	2.27 ± 0.3 (6)
0–21 day	2.79 ± 0.4[c] (3)	—	—
Pregnant rats			
7-day	6.83 ± 0.9 (9)	5.60 ± 0.8[c] (9)	1.95 ± 0.5 (9)
14-day	5.89 ± 0.9 (8)	2.39 ± 0.3 (15)	3.03 ± 0.5 (8)
21-day	6.52 ± 0.7 (12)	1.90 ± 0.3 (9)	1.62 ± 0.6 (12)
Exer. + pregnancy			
0– 6 day	5.30 ± 0.5 (9)	—	—
7–13 day	6.84 ± 0.7 (9)	—	—
14–20 day	3.05 ± 0.5[c,d] (12)	1.06 ± 0.3[c,f] (9)	2.19 ± 0.2 (12)
0–20 day	4.95 ± 0.5[e] (9)	—	—

[a] Mean ± SEM; ( ) = number of observations, *i.e.*, gut segments.
[b] $p < .05$ when compared with virgin controls.
[c] $p < .01$ when compared with virgin controls.
[d] $p < .01$ when compared with 21-P rats.
[e] $p < .02$ when compared with comparable virgin rats and the 14-20PE rats.
[f] $p < .05$ when compared with 21-P rats.

rats for 21 days did not alter gut transport of alanine when these rats are compared with virgin control rats. In contrast, the 0-20PE rats definitely displayed the effects of pregnancy in increasing transport of alanine when compared with the 0–21-day exercised virgin group ($p<.02$) and the effects of extended exercise when compared with the 14-20PE group ($p<.02$). Rats exercised the last week of pregnancy appeared to transport alanine and lysine to a lesser extent than nonexercised pregnant rats ($p<.01$, $<.05$, for 21-P *vs* 14-20PE).

Discussion. The demands of chronic exercise and pregnancy on maternal substrates are similar in many ways, though each is oriented toward different physiological goals. Thus, chronic exercise necessitates greater provision of amino acids to support the well-known skeletal muscle hypertrophy which attends running. Contrarily, evidence is available indicating that skeletal muscle protein depots are mobilized to support embryonic growth during relative protein depletion or stressful situations (7). Therefore, the combined stressor effects of exercise and pregnancy present conflicting and simultaneous demands of structural protein (anabolism *vs* catabolism) to the skeletal muscle. With the exception of lysine transport in the 7-day pregnant rat, the absence of effects of pregnancy on gut transport of three amino acids (per unit gut weight) studied herein agree with *in vivo* studies employing methionine and glycine (4, 5). These results may be interpreted in two ways, namely, that morphological alterations (thickening of mucosal lining, increased villi dimensions, *etc.*) known to occur with pregnancy may offset (quantitatively) increased amino acid transport to meet embryonic needs; alternatively, pregnancy simply does not alter gut transport of amino acids on a per unit gut weight basis. Such considerations do not obviate the possibility that elongation of the gut during pregnancy coincident with constant amino acid transport rate would result in increased absorptive capacity of the total gut.

Decreases in net accumulation of alanine and lysine due to exercise during the last week of pregnancy were somewhat surprising even in recognition of the fact that gut absorption of amino acids obviously must antecede any metabolic rearrangements at the skeletal muscle level. In contrast to the first 15 days of pregnancy when fatty acid substrates are deposited in large amounts, the last 7 days of the gestational period appear to be associated with prolific nitrogen conservation and protein deposition at the expense of some maternal muscle stores (7–10). Thus, the decrease in transport of alanine and lysine seen in the 14-20PE groups is somewhat in contrast to that which would be expected unless the maternal catabolic processes are so efficient in delivering protein precursors from maternal stores that intestinal provision of these materials can be decreased concomitantly. Undoubtedly, the increased circulating ovarian steroids, ACTH, and glucocorticoids during pregnancy (11, 12) contribute to this compensation mainly by the latter hormone's depression of muscle protein synthesis while increasing degradation of depot-protein as has been shown by labeled-amino acid studies (7, 13). Such activity decreases the need for high levels of intestinal transport of amino acids.

Finally, it would appear that the nutritional requirements effected by pregnancy offset the deleterious effects of 21 days of exercise on gut translocation of amino acids. The rat, therefore, appears better able to compensate for the dual need for amino acids inflicted by exercise and pregnancy if exercise is induced early in the gestational period and maintained throughout to term.

Summary. Amino acid transport by everted gut segments (*in vitro*) of rats exercised 7 or 21 days, rats pregnant for 7, 14, or 21 days, or rats exercised during different periods of gestation was determined. Generally, pregnancy did not affect amino acid transport; only exercise during the last week of pregnancy had a negative effect on gut transport.

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