

Urea kinetics in healthy women during normal pregnancy

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Urea kinetics were measured in normal women aged 22–34 years at weeks 16, 24 and 32 on either their habitual protein intake (HABIT) or a controlled intake of 60 g protein/d (CONTROL), using primed-intermittent oral doses of [¹⁵N¹⁵N]urea and measurement of plateau enrichment in urinary urea over 18 h (ID) or a single oral dose of [¹⁵N¹⁵N]urea and measurement of enrichment of urea in urine over the following 48 h (SD). The intake of protein during HABIT-ID (80 g/d) was greater than that on HABIT-SD (71 g/d); urea production as a percentage of intake was significantly greater at week 16 for HABIT-ID than HABIT-SD, whereas urea hydrolysis at week 16 was greater for HABIT-SD than HABIT-ID and urea excretion at week 32 was greater for HABIT-ID than HABIT-SD. The combined results for HABIT-ID and HABIT-SD showed a significant reduction in urea production at week 32 compared with week 24. Urea excretion decreased significantly from week 16 to week 24 with no further decrease to week 32 and urea hydrolysis was significantly greater at week 24 than either week 16 or week 32. Compared with HABIT, on CONTROL there was a decrease in urea production at week 16, and urea excretion was significantly reduced at week 16. For all time periods urea production was closely related to the sum of intake plus hydrolysis. Hydrolysis was greatest at week 24 and closely related to urea production. There was a significant inverse linear relationship overall for hydrolysis as a proportion of production and excretion as a proportion of intake. The results show that on HABIT N is more effectively conserved in mid-pregnancy through an increase in urea hydrolysis and salvage, and during late pregnancy through a reduction in urea formation. Lowering protein intake at any stage of pregnancy increased the hydrolysis and salvage of urea. The staging of these changes was later than that in pregnancy in Jamaica.

[¹⁵N]urea: Urea kinetics: Protein requirements: Pregnancy

For a mother to be able to sustain a pregnancy successfully requires that she is able to satisfy the nutritional and metabolic needs of her own body and that of the foeto-placental unit. Extreme nutritional deprivation, either before or during pregnancy can limit the ability of the embryo and fetus to grow (Kramer, 1987; Rush, 1989). However, the extent to which more modest differences in maternal size or body composition at the start of pregnancy, or nutritional intake during pregnancy, might compromise either fetal growth or the functional capacity of the offspring is less clear (National Academy of Sciences, 1990; Barker, 1992). The definition of nutrient requirements during pregnancy in the human subject is inherently difficult. First, there are relatively small changes over relatively long periods of time. Second, in the short term, changes in maternal body composition might effectively buffer any effect of modest dietary influences. Consequently there is uncertainty in setting recommendations for dietary intakes during pregnancy (Department of Health and Social Security, 1979; Food and Agriculture Organization/World Health Organization/United Nations University, 1985; Committee on Medical Aspects of Food Policy, 1991). The use of balance studies to determine requirements is not adequate because although balance has to be achieved in order to identify a diet as being adequate, balance of itself is

not a sufficient criterion for the determination of adequacy (Rand *et al.* 1984; Food and Agriculture Organization/World Health Organization/United Nations University, 1985; Committee on Medical Aspects of Food Policy, 1991). In response to this difficulty increased use has been made of more dynamic measures of amino acid and protein metabolism to characterize overall metabolic well-being (Young *et al.* 1989; Millward, 1992; Jackson, 1995).

For pregnancy there is relatively little information available (Hyttén & Leitch, 1971; Calloway, 1974; National Academy of Sciences, 1990). The needs for protein for net deposition in maternal tissues and the growth of the fetus and placenta are not distributed evenly throughout pregnancy and animal studies indicate preferential deposition of maternal protein early in pregnancy, which potentially can be utilized by the placenta and fetus at a later stage (Naismith & Morgan, 1976; Mayel-Afshar & Grimble, 1983).

In a normal pregnancy the average weight gain is about 12.5 kg of which 0.9 kg is protein, giving an average gain of 3 g/d or 480 mg N/d (Hyttén & Leitch, 1971). Balance studies indicate a positive daily balance of 1–3 g N/d during late pregnancy (King, 1975; Johnstone *et al.* 1981). Positive balance is associated with a reduction in the rate of urea loss in urine, which has been presumed to reflect a decrease in urea production. However, changes in urea excretion may not necessarily reflect changes in urea production because when the body is seeking to economize on N there may be an increase in the salvage of urea-N following enhanced hydrolysis of urea by the colonic microflora (Langran *et al.* 1992; Jackson, 1993). Urea production has been measured at some time during pregnancy in three studies (Kalhan *et al.* 1982; Olufemi *et al.* 1991; Forrester *et al.* 1994). Forrester *et al.* (1994) measured urea kinetics longitudinally in pregnant Jamaican women and found that protein intake did not change during pregnancy, but urea hydrolysis was significantly increased early in pregnancy, falling to levels similar to the non-pregnant state by the third trimester. In the other two studies urea production in the third trimester was 40 % lower than postpartum when measured towards the end of a 17 h fast (Kalhan *et al.* 1982) or in the post-absorptive state (Olufemi *et al.* 1991). These studies suggest that any changes in late gestation might be relatively modest, without precluding more marked changes earlier in pregnancy (Forrester *et al.* 1994).

In the present study urea kinetics have been measured longitudinally in women on their habitual dietary intake at 16, 24 and 32 weeks gestation compared with women in whom protein intake was controlled at 60 g protein/d, the recommended daily amount (RDA) at the time the studies were carried out (Department of Health and Social Security, 1979). Although scientifically it might have been desirable to reduce the protein intake to the physiological minimum requirement, about 40 g protein/d, this was not acceptable ethically.

METHODS

Subjects

The studies were carried out in eighteen women aged 22–34 years. Sixteen of the eighteen women were recruited from the antenatal booking clinic of the Princess Anne Hospital, Southampton and two were recruited through personal contact. The studies had the approval of the Southampton Hospitals and South West Hampshire Health Authority Ethical Subcommittee. The women agreed to participate after the nature of the investigations had been explained to them. All the women were in good health with no obvious medical problems or complications of pregnancy. They had been consuming a normal diet and none had been taking any form of medication before recruitment. During

Table 1. The age, parity and gestational weight of eighteen women who participated in one of two protocols to measure urea kinetics, with the gender and weight of the newborn infant

Subject no.	Age (years)	Primiparous	Wt at 16 weeks (kg)	Wt gain weeks 16 to 24 (kg)	Weight gain weeks 24 to 32 (kg)	Protocol	Sex of infant	Birth wt (g)
1	29	YES	56.0	5.0	3.5	1	M	3450
2	29	NO	62.7	1.7	2.7	1	F	3060
3	32	YES	60.1	5.0	7.1	1	M	3900
4	31	NO	56.0	5.0	5.5	1	F	3200
5	30	NO	54.4	4.1	3.1	1	M	2680
6	22	NO	68.2	4.8	-0.8	1	F	3940
7	28	NO	68.0	5.5	3.2	1	F	3970
8	23	YES	70.4	8.0	6.4	1	M	3420
9	26	YES	64.1	8.3	6.2	1	M	3680
10	26	YES	60.0	0	3.0	1	M	2980
11	27	YES	54.6	1.6	6.6	2	M	3495
12	33	NO	57.2	2.8	2.8	2	M	2200
13	34	NO	63.0	5.0	2.2	2	F	4045
14	26	YES	66.2	6.0	3.8	2	F	4000
15	30	NO	75.6	6.8	6.4	2	M	3510
16	28	YES	61.8	6.0	3.8	2	M	3280
17	33	NO	66.4	3.0	1.2	2	F	4325
18	28	YES	61.8	3.6	2.2	2	M	3170

Protocol 1, primed intermittent doses of labelled urea; protocol 2, single doses of labelled urea.

their pregnancies some women were prescribed medication for indigestion or 'heartburn' whilst others were prescribed Fe tablets or injections. No constraints were placed on the behaviour of the women during the studies, which were carried out in their own homes. The characteristics of the subjects and protocols in which they participated are shown in Table 1.

Protocols

The objective of the study was to measure urea kinetics at three different stages of pregnancy on the women's habitual protein intake and to compare these results with urea kinetics in women in whom dietary intake of protein was controlled at 60 g/d (Department of Health and Social Security, 1979). At the end of the first study it was clear that the variation in urea kinetics between women was sufficiently great that it might be difficult to identify differences on a group basis on the 60 g protein/d intake. Therefore, the second part of the study was designed as an intervention study in which urea kinetics were measured in the same women before and after taking the controlled protein intake. This placed greater demands on their participation and they identified a preference for a less intrusive approach to the measurement of urea kinetics in which a single dose of labelled urea was taken and urine collected for a period of 48 h (Jackson *et al.* 1993).

Protocol 1: primed intermittent doses of labelled urea. The objective was to determine urea kinetics longitudinally throughout pregnancy in women taking their habitual intake of energy and protein (HABIT-ID). The women completed a questionnaire and a 2 d dietary recall to provide information on food preferences, the typical pattern of food consumption and an estimate of habitual intake. Dietary analysis for individual macronutrient consumption was carried out using the COMPEAT computer package (Nutrition Service

Ltd, London). For the day before the study and the day of the study itself food was provided to the women. All food was prepared and delivered to the subjects' homes. The food was provided as five isonitrogenous meals daily, and was composed of ordinary foodstuffs. On day 1 the women ate the food at their preferred meal times but on day 2 the food was consumed at intervals of 3 h from 06.00 hours. During the final 24 h of each study period urea kinetics were measured using the primed-intermittent presentation of [$^{15}\text{N}^{15}\text{N}$]urea (99 % atoms ^{15}N ; Cambridge Isotope Laboratories, Andover, MA, USA). An accurately known amount of [$^{15}\text{N}^{15}\text{N}$]urea was made up in sterile water and kept on ice until ready for use. At midnight a priming dose of isotopic urea equivalent to 15 h of intermittent doses (28.5 mg) was given orally to shorten the time taken to achieve a plateau in isotopic enrichment in urinary urea. From 06.00 hours single doses of urea (5.5 mg) were administered at 3 h intervals until 15.00 hours. Urine was collected from 21.00 hours to midnight, immediately before the administration of the first dose of isotope, for the measurement of background enrichment, between midnight and 06.00 hours and at 3 h intervals from 06.00 hours until 21.00 hours.

Protocol 2: single doses of labelled urea. The objective was to determine urea kinetics longitudinally throughout pregnancy in women taking a controlled intake of 60 g protein/d. An estimate of the habitual energy and protein intake and the amount and type of food was made for each woman using a 1 d dietary recall while information on food preferences was obtained by questionnaire. The study period lasted for 7 d. During the first 48 h urea kinetics were measured using the single-dose method, while the women were consuming their habitual intake and an accurate written record was kept of their actual *ad libitum* intake (HABIT-SD). The women were then placed on the controlled diet, designed to provide their habitual intake of energy and 60 g protein/d for 6 d. Urea kinetics were measured again during the final 48 h period using the single-dose method (CONTROL-SD). The test diets were prepared from ordinary foodstuffs and were delivered to the women in their homes. If during the study, the women developed a dislike for any of the foods an alternative of similar protein composition was provided. Measurements of urea kinetics were made with the oral presentation of a single dose of [$^{15}\text{N}^{15}\text{N}$]urea (99 % atoms ^{15}N ; Cambridge Isotope Laboratories). The [$^{15}\text{N}^{15}\text{N}$]urea was made into solution using approximately 23 ml sterile water and kept on ice until ready for use. A specimen of urine was taken for the measurement of baseline enrichment and the [$^{15}\text{N}^{15}\text{N}$]urea, 100 mg, was taken orally at 09.00 hours. All urine passed in the following 48 h was collected in two portions of 24 h each.

Urea kinetics

Analyses. All specimens of urine were collected into acidified containers and stored frozen until analysed. The concentration of urea and NH_3 in urine was measured using the Berthelot method (Kaplan, 1965) and urea-N was isolated from urine for mass spectrometry using short ion-exchange column chromatography (Jackson *et al.* 1980). N_2 gas was liberated from urea by reaction with alkaline hypobromite. In this reaction N is released from urea in a monomolecular reaction (Walser *et al.* 1954), hence the relative proportions of [$^{15}\text{N}^{15}\text{N}$]urea, [$^{15}\text{N}^{14}\text{N}$]urea and [$^{14}\text{N}^{14}\text{N}$]urea can be determined. Measurements of enrichment were carried out in a triple-collector isotope-ratio mass spectrometer (SIRA 10; VG Isogas, Winsford, Ches.).

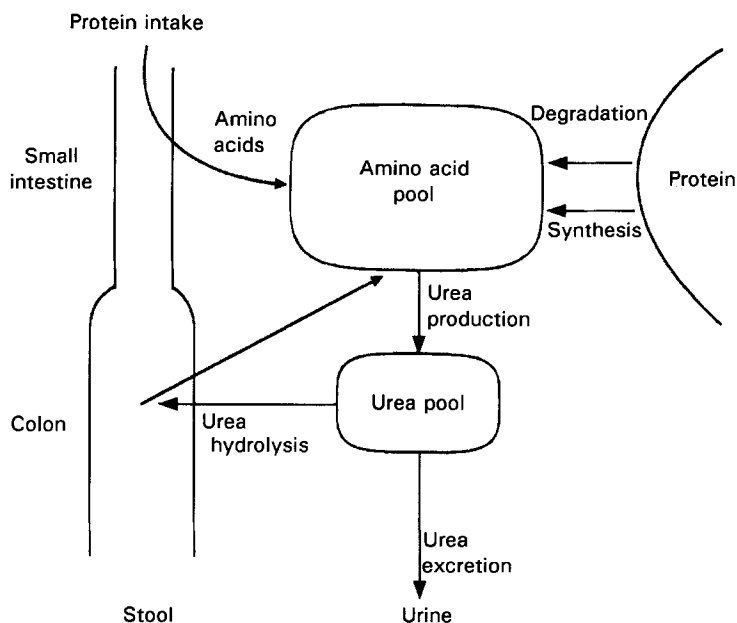


Fig. 1. Urea kinetics are characterized within a metabolic model which consists of the exchanges and interaction amongst an amino acid pool, a protein pool and a urea pool. Amino acids derived from ingested protein contribute to the amino acid pool, which also receives inputs from protein degradation and the salvage of urea-nitrogen. Amino acids are either used for protein synthesis or oxidized with the formation of urea. A portion of the urea produced is excreted in urine and a portion passes to the colon where it is hydrolysed by the metabolic activity of the colonic microflora.

Calculations. The model of protein, amino acid and N metabolism within which urea kinetics are determined is shown in Fig. 1.

For protocol 1 (primed intermittent doses of labelled urea) urea kinetics were calculated by the model of Jackson *et al.* (1984). Ingested protein is made available to metabolism as amino acids, which mix with the general body pool; amino acids also derive from protein degradation. Once an isotopic steady-state has been achieved, the dilution of an intermittent dose of [$^{15}\text{N}^{15}\text{N}$]urea gives a measure of the rate of urea appearance in the urea pool, that is the rate of urea production in the body. Only a proportion of the urea produced is excreted in the urine. The difference between the urea produced and that excreted is presumed to have been hydrolysed in the bowel with the N being returned to the general metabolic pool of N and being available for further metabolic interaction. Details of the calculations are given by Jackson *et al.* (1984).

For protocol 2 (single doses of labelled urea) urea kinetics were calculated by the model of Jackson *et al.* (1993). A known amount of isotopically-labelled [$^{15}\text{N}^{15}\text{N}$]urea mixes freely with the urea pool of the body and is presumed to trace reliably the fate of the urea in that pool. The labelled urea is cleared from the pool in 36–48 h and the proportion of the dose lost in the urine is similar to the proportion of endogenously formed urea which is lost in the urine. From a knowledge of the amount of urea in the urine and the proportion of the labelled urea lost in the urine, the rate of endogenous urea formation can be calculated and by difference the amount of urea hydrolysed by the colonic microflora can be derived. Details of the calculations are given by Jackson *et al.* (1993).

The assumption is made that an oral dose of [$^{15}\text{N}^{15}\text{N}$]urea is absorbed intact, without any hydrolysis of the label before absorption. This assumption is not justified in individuals

in whom there is gastric infection with *Helicobacter pylori* (Graham *et al.* 1987). The hydrolysis of the dose before absorption can be determined by measuring the ratio of the rate at which labelled urea-N derived from urea hydrolysis is returned to urea formation, relative to the measured rate of urea formation (Hibbert *et al.* 1992). The assumption is considered not to hold when this ratio exceeds 15 % (Hibbert *et al.* 1992), and in two studies the data were excluded from analysis because this condition applied and they were rejected as unsatisfactory.

Comparisons between groups of data were carried out using ANOVA and paired *t* tests as appropriate. Results are reported as means and standard deviations. Although results might usually be expressed as mg N/kg per d, this presents problems in pregnancy when there are complex changes in weight and body composition (Thompson & Halliday, 1992). There is no completely satisfactory way around this difficulty. In order to reduce any chance of misinterpretation the results have been expressed in relation to the weight of each subject at the time of the first study.

RESULTS

The results are reported for the studies which were completed satisfactorily and represent a total of fifty-four measurements of urea kinetics made in eighteen women. Not all women were able to complete the entire series and ten women have been excluded from the analysis either for this reason or because of clinical complications. All the women who completed the studies had normal pregnancies and gave birth to babies with a mean birth weight of 3461 (SD 536) g (Table 1). The women accepted the diets and there were no major problems with compliance. Great emphasis was placed on the importance of complete urine collections. We were not willing to give pregnant women a marker to validate the completeness of urine collections, but based on the measurement of urinary creatinine (data not shown) we have no reason to believe that there were any failures which would seriously prejudice the data or the interpretations.

Protocol 1: primed intermittent doses of labelled urea

There were five primiparous and five multiparous women in the group and the average ages were 27 and 28 years respectively (Table 1). At week 16 of gestation both the primiparous women and the multiparous women weighed 62 kg on average. Gains in weight between week 16 and week 24 were 5.3 and 5.2 kg for the primiparous and multiparous women respectively and between 24 and 32 weeks 5.2 and 2.7 kg. The average weight of the infants for the primiparous women was 3486 g and for the multiparous women 3370 g. There were five male and five female offspring in the group.

The levels of plateau enrichments in urinary urea were identified by visual inspection and a satisfactory plateau was achieved in all the studies. The coefficient of variability for the enrichment in mass 30:28 at plateau was 14 % on average (range 2–26 %). Table 2 shows the results for urea kinetics for the women at weeks 16, 24 and 32 of pregnancy. There were no significant differences in protein intake between trimesters (13.3, 12.9, 12.8 g N/d, approximately 80 g protein/d), nor in urea production. Urinary excretion of urea demonstrated a phasic response. Excretion at week 24 was significantly less than at week 16, a 25 % reduction. There was an increase in excretion between week 24 and week 32, which did not achieve statistical significance. The largest differences between the different stages of pregnancy were seen for urea hydrolysis. There was a statistically significant increase from week 16 to week 24 (190 %) and a statistically significant fall from week 24 to week 32, with the result that there was no difference between week 16 and

Table 2. Urea kinetics (mg nitrogen/kg per d) were measured in ten women by primed intermittent oral doses of [$^{15}\text{N}^{15}\text{N}$]urea at weeks 16, 24 and 32 of pregnancy whilst they were consuming their habitual intake of 80 g protein/d (HABIT-ID)

Week of pregnancy ... Subject no.	N intake			Urea production			Urea excretion			Urea hydrolysis			N intake plus urea hydrolysis		
	16	24	32	16	24	32	16	24	32	16	24	32	16	24	32
1	329	339	339	254	377	248	192	151	211	62	226	37	391	565	376
2	195	112	97	195	123	112	125	59	55	70	64	58	265	176	155
3	214	236	226	192	190	182	128	105	118	64	85	65	278	321	291
4	218	208	208	199	194	218	161	129	146	38	65	72	256	273	280
5	236	235	235	200	212	211	140	106	152	60	106	59	296	341	294
6	172	288	243	203	209	207	159	83	138	44	126	69	216	414	312
7	203	167	167	169	177	201	132	94	134	37	83	68	240	250	235
7	193	138	161	142	157	168	113	89	155	30	68	13	223	206	174
9	204	176	212	180	168	131	142	86	90	38	81	42	242	257	254
10	174	176	189	178	180	143	131	159	96	47	21	47	221	197	236
Mean	214	208	208	191	199	182	142	106**	129	49	93*	53†	263	300	261
SD	45	69	63	29	68	43	23	32	42	14	54	18	52	118	65

Mean values were significantly different from those at 16 weeks: * $P < 0.05$, ** $P < 0.01$.

Mean value was significantly different from that at 24 weeks: † $P < 0.05$.

Table 3. Urea kinetics were measured at week 16, week 24 and week 32 of pregnancy in women who were taking their habitual protein intake, either with primed intermittent oral doses of [$^{15}\text{N}^{15}\text{N}$]urea (HABIT-ID), or a single oral dose of [$^{15}\text{N}^{15}\text{N}$]urea, either on their habitual intake of protein (HABIT-SD) or a restricted intake of 60 g/d (CONTROL-SD)§

	Production: intake (%)	Excretion: intake (%)	Excretion: production (%)	Hydrolysis: production (%)
Week 16				
HABIT-ID	91	68	75	26
HABIT-SD	121*	70	60	40
CONTROL-SD	101	57	56	44
Week 24				
HABIT-ID	98	54	55†††	45†††
HABIT-SD	122	57	45	55
CONTROL-SD	114	51	49	51
Week 32				
HABIT-ID	91	64	70‡‡	30‡‡
HABIT-SD	93	50	56	44
CONTROL-SD	111	52	48	52

Mean value was significantly different from that for HABIT-ID (ANOVA and *post hoc t* test): * $P < 0.05$.

Mean values were significantly different from those at week 16 (ANOVA and *post hoc t* test): ††† $P < 0.001$.

Mean values were significantly different from those at week 24 (ANOVA and *post hoc t* test): ‡‡ $P < 0.01$.

§ For details of subjects and procedures, see Table 1 and pp. 166–170.

week 32. The relationship between protein intake and urea production remained fairly constant throughout pregnancy, with production being 91–98 % of N intake (Table 3). Urea excretion was 68 % of intake at week 16 of pregnancy, falling significantly to 54 % of intake at week 24 and increasing at week 32 when it was 64 % of intake. However, there were substantial differences in the disposal of urea between excretion and hydrolysis, with hydrolysis being 26 % of production at week 16, increasing to 45 % of production at week

24 and falling to about 30 % of production by week 32. The groups were too small to identify differences in kinetics in relation to parity, weight gain or weight of the offspring.

Protocol 2: single doses of labelled urea

There were four primiparous and four multiparous women in the group and the average ages were 27 and 32.5 years respectively (Table 1). At 16 weeks of gestation the primiparous women weighed 61 kg and the multiparous women weighed 66 kg on average. Gains in weight between weeks 16 and 24 were 4.3 and 4.4 kg for the primiparous and multiparous women respectively and between 24 and 32 weeks the corresponding values were 4.1 and 3.1 kg. The average weight of the infants for the primiparous women was 3486 g and for the multiparous women 3520 g. There were five male and three female offspring in the group.

Initially urea kinetics were measured when the women were on their habitual protein intake (HABIT-SD) and then again after taking a restricted intake of protein (60 g/d) for 5 d (CONTROL-SD). Urea kinetics were measured on each occasion by giving a single oral dose of labelled urea and following the excretion of label in urine over 48 h. The habitual intake of protein was not different between trimesters (11.2, 12.3, 10.9 g N/d, 71 g protein/d on average; Table 4).

On HABIT-SD, urea production did not change between week 16 and week 24, but there was a statistically significant decrease from week 24 to week 32 of about 30 %. Urea excretion decreased progressively from week 16 to week 32, by 28 %, and the difference between week 16 and week 32 was statistically significant. Urea hydrolysis was greatest at week 24, 135 and 175 % of week 16 and week 32 respectively, and the difference between week 24 and week 32 was highly statistically significant.

On the CONTROL-SD (60 g protein/d) there were no differences in urea production or urea excretion between trimesters. Urea hydrolysis was greatest at week 24, but the

Table 4. Urea kinetics (mg nitrogen/kg per d) were measured at weeks 16, 24 and 32 of pregnancy in eight women using a single oral dose of [$^{15}\text{N}^{15}\text{N}$]urea, while they were taking their habitual protein intake of 71 g/d (HABIT-SD), and again after 5 d on a diet which provided 60 g protein/d (CONTROL-SD)§

(Values are means and standard deviations)

	N intake		Urea production		Urea excretion		Urea hydrolysis		N intake plus urea hydrolysis	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Week 16										
HABIT-SD	176	46	204	53	121	41	83	41	259	54
CONTROL-SD	153	15	155	39	87†	24	68	28	221	39
Week 24										
HABIT-SD	195	60	224	36	102	41	120	32	316	74
CONTROL-SD	153	15	175	67	76	28	98	77	251	84
Week 32										
HABIT-SD	175	35	159***	43	87†	27	72***	37	247	54
CONTROL-SD	153	15	168	57	79	27	90	49	243	49

Mean values were significantly different from those at week 24 (ANOVA and *post hoc t* test): *** $P < 0.001$.

Mean value was significantly different from that at week 16 (ANOVA and *post hoc t* test): † $P < 0.05$.

Mean value was significantly different from that for HABIT-SD (ANOVA and *post hoc t* test): ‡ $P < 0.05$.

§ For details of subjects and procedures, see Table 1 and pp. 166–170.

differences between the different periods were relatively small and none were statistically significant.

Comparison between protocol 1 (the intermittent-dose method) and protocol 2 (the single-dose method)

The results for urea kinetics in HABIT-ID were compared with HABIT-SD. The groups were very similar in terms of weight and weight changes during pregnancy. Although the HABIT-SD subjects ingested less protein, the difference was not statistically significant, but urea production as a percentage of N intake was significantly higher for HABIT-SD at week 16 ($P = 0.042$). The only other differences in any aspect of urea kinetics between the two groups were in urea hydrolysis at week 16 and urea excretion at week 32. At week 16 urea hydrolysis was significantly greater for HABIT-SD than HABIT-ID ($P = 0.026$). During week 32 urea excretion was significantly greater for HABIT-ID than HABIT-SD ($P = 0.027$). These differences probably reflect the differences in protein intake. When HABIT-ID and HABIT-SD were combined (Table 5) there was a significant decrease in urea production at week 32 compared with week 24 ($P = 0.03$) and a marginal reduction compared with week 16 ($P = 0.08$). Urea excretion was significantly reduced at week 24 ($P = 0.017$) and marginally at week 32 ($P = 0.079$) compared with week 16. There was a significant increase in urea hydrolysis at week 24 compared with either week 16 ($P = 0.0049$) or week 32 ($P = 0.0024$).

Comparison between habitual and controlled protein intake

For CONTROL-SD at 16 weeks, urea production was reduced compared with HABIT-SD at a marginal level of statistical significance ($P = 0.058$); however, there was a statistically significant reduction in urea excretion for CONTROL-SD ($P = 0.0094$). Differences of

Table 5. Comparison of urea kinetics in ten non-pregnant and eighteen pregnant women taking either their habitual intake of protein, or the recommended daily allowances (Department of Health and Social Security, 1979) of protein of 55 g/d for non-pregnant women (data from McClelland & Jackson, 1996) and 60 g/d for pregnant women§

	Habitual protein intake				Controlled protein intake			
	Non-pregnant	Week 16	Week 24	Week 32	Non-pregnant	Week 16	Week 24	Week 32
Intake (mg N/kg per d)	181	197	202	193	153	153**	153**	153**
Urea production (mg N/kg per d)	200	197	210	172†††	186	155**	175	168
Urea excretion (mg N/kg per d)	124	133	105††	110†	111	87***	76*	79*
Urea hydrolysis (mg N/kg per d)	76	64	105†††	62†††	74	68	98	90*
Production (% intake)	112	104	108	92	122	101	114	111
Excretion (% production)	62	68	51	63	59	56	49**	48**

Mean values were significantly different from those of the equivalent stage of pregnancy on the habitual protein diet (unpaired *t* test): * $P < 0.08$, ** $P < 0.05$, *** $P < 0.01$.

Mean values were significantly different from those at week 16: † $P < 0.08$, †† $P < 0.05$, ††† $P < 0.01$.

Mean values were significantly different from those at week 24: †† $P < 0.05$, ††† $P < 0.01$.

§ For details of subjects and procedures, see Table 1 and pp. 166–170.

borderline statistical significance were seen for urea production ($P=0.07$) and urea excretion ($P=0.047$) between the two diets at week 24. There were no statistically significant differences in urea production and excretion at week 32. There were no differences in urea hydrolysis between the two diets at weeks 16, 24 or 32.

When CONTROL-SD was compared with all the women on their habitual intake (HABIT-ID and HABIT-SD; Table 5) urea production was significantly less on CONTROL-SD at week 16 ($P=0.021$), and urea excretion was significantly less at week 16 ($P=0.0015$), and marginally less at week 24 ($P=0.053$) and week 32 ($P=0.062$). Urea hydrolysis was marginally higher on CONTROL-SD at week 32 ($P=0.075$). The results were compared with those obtained in non-pregnant women consuming their habitual diet or a diet in which the protein content was restricted to 55 g/d (McClelland & Jackson, 1996). There was very little difference in the pattern of response to the 55 g protein/d diet in the non-pregnant women and the changes seen at week 16 in the pregnant women on 60 g protein/d.

For every aspect of urea kinetics there was considerable variation between individuals. There are a number of factors which might have contributed towards this variability, but the groups were too small to identify their possible contribution with any confidence. However, the protein intake of HABIT-SD was between that of HABIT-ID and CONTROL-SD so taken together the studies represented a range of intakes from about 60 g protein/d to 80 g protein/d. The relationships between different aspects of urea kinetics were determined for the group as a whole for each of the periods of pregnancy (Table 6). The impression was obtained of a similarity in response at week 16 and week 32 which was different from week 24. Intake was more strongly related to excretion at weeks 16 and 32, but more strongly related to production and hydrolysis at week 24. Production was more strongly related to excretion at week 16 and week 32, but more strongly related to hydrolysis at week 24. At each of the time points intake plus hydrolysis was very strongly related to production and this relationship was stronger than that between intake

Table 6. Relationship between different aspects of urea kinetics in pregnant women determined by linear regression analysis*

	Week of gestation	<i>r</i>	Statistical significance: <i>P</i>	Slope
Intake v. production	16	0.45	0.02	0.47
	24	0.61	0.001	0.58
	32	0.48	0.14	0.50
Intake v. excretion	16	0.67	0.0001	0.83
	24	0.37	0.06	0.61
	32	0.73	< 0.0001	0.89
Intake v. hydrolysis	16	-0.16	0.43	
	24	0.42	0.03	0.44
	32	-0.19	0.36	
Production v. excretion	16	0.72	< 0.0001	0.85
	24	0.41	0.04	0.71
	32	0.64	0.0004	0.74
Production v. hydrolysis	16	0.56	0.003	0.79
	24	0.82	< 0.0001	0.89
	32	0.56	0.003	0.70
Intake plus hydrolysis v. production	16	0.75	< 0.0001	0.87
	24	0.84	< 0.0001	1.34
	32	0.80	< 0.0001	0.95

*For details of subjects and procedures, see Table 1 and pp. 166-170.

and production. In Fig. 2, the relationship between production and either intake or intake plus hydrolysis has been shown for HABIT-ID, HABIT-SD and CONTROL-SD. It can be seen that there was a more consistent close linear relationship between production and intake plus hydrolysis, than between production and intake alone. Fig. 3 shows that for each of the three studies, HABIT-ID, HABIT-SD, CONTROL-SD there was a significant inverse linear relationship between the proportion of production which was hydrolysed and the proportion of intake which was excreted.

DISCUSSION

In the present study urea kinetics have been measured in normal pregnant women at different times during pregnancy and the response to a controlled dietary intake of protein has been observed. The controlled intake, 60 g protein/d, represented a relatively modest restraint, but was set at the RDA for protein at the time the study was developed (Department of Health and Social Security, 1979). More recently the reference nutrient intake for protein during pregnancy has been set at a lower level, 51 g/d (Committee on Medical Aspects of Food Policy, 1991). The habitual protein intake for the women in HABIT-ID was 80 g/d and for women in HABIT-SD 71 g/d. There is considerable variability between individuals of about 25 % for most aspects of protein and N metabolism and this will tend to obscure differences between groups even where real differences might exist. It was for this reason that urea kinetics were measured twice in the same woman for HABIT-SD and CONTROL-SD. Two methods have been used for measuring urea kinetics. Primed intermittent oral doses and a single oral dose have been compared directly in the same subjects under the same conditions and have been found to give similar results (Jackson *et al.* 1993). In the present study there were differences in the values for urea kinetics between HABIT-ID and HABIT-SD; however, the differences were most probably the result of the differences in protein intake between the two groups of women.

When the data for all the women on the habitual and controlled intake are considered together (Tables 5 and 6, Fig. 2) the pattern of response in urea kinetics during pregnancy appears to consist of two components. On the habitual diet, at week 16, urea production, excretion and hydrolysis were not different from corresponding values for the non-pregnant state. At week 24, urea production was unchanged but a smaller proportion of production was excreted, with an increase in the proportion hydrolysed. At week 32 urea production was reduced compared with either week 16 or week 24 and was associated with a fall in urea hydrolysis compared with week 24, to a level similar to that at either week 16 or the non-pregnant state. The response to an intake of 60 g protein was a modest reduction in urea production, most obvious at week 16, with relatively less of urea production being excreted and more being hydrolysed at each stage of pregnancy compared with the habitual intake.

There are two points at which control might be exerted over the fate of amino acids and N within the mother's body. First, there is the partitioning of amino acids between pathways which lead either to protein synthesis or to amino acid oxidation with the formation of urea (Fig. 1). The best expression we have of the relative flow to these two possible fates is urea production : N intake (Table 3, Figs 2 and 4). Second, the N which moves to urea formation has one of two possible fates determined by the partitioning of urea between urinary excretion and hydrolysis in the bowel with salvage of the N for further metabolic interaction. The best expressions we have of the relative flow to these possible fates is urea excretion : urea production and urea hydrolysis : urea production

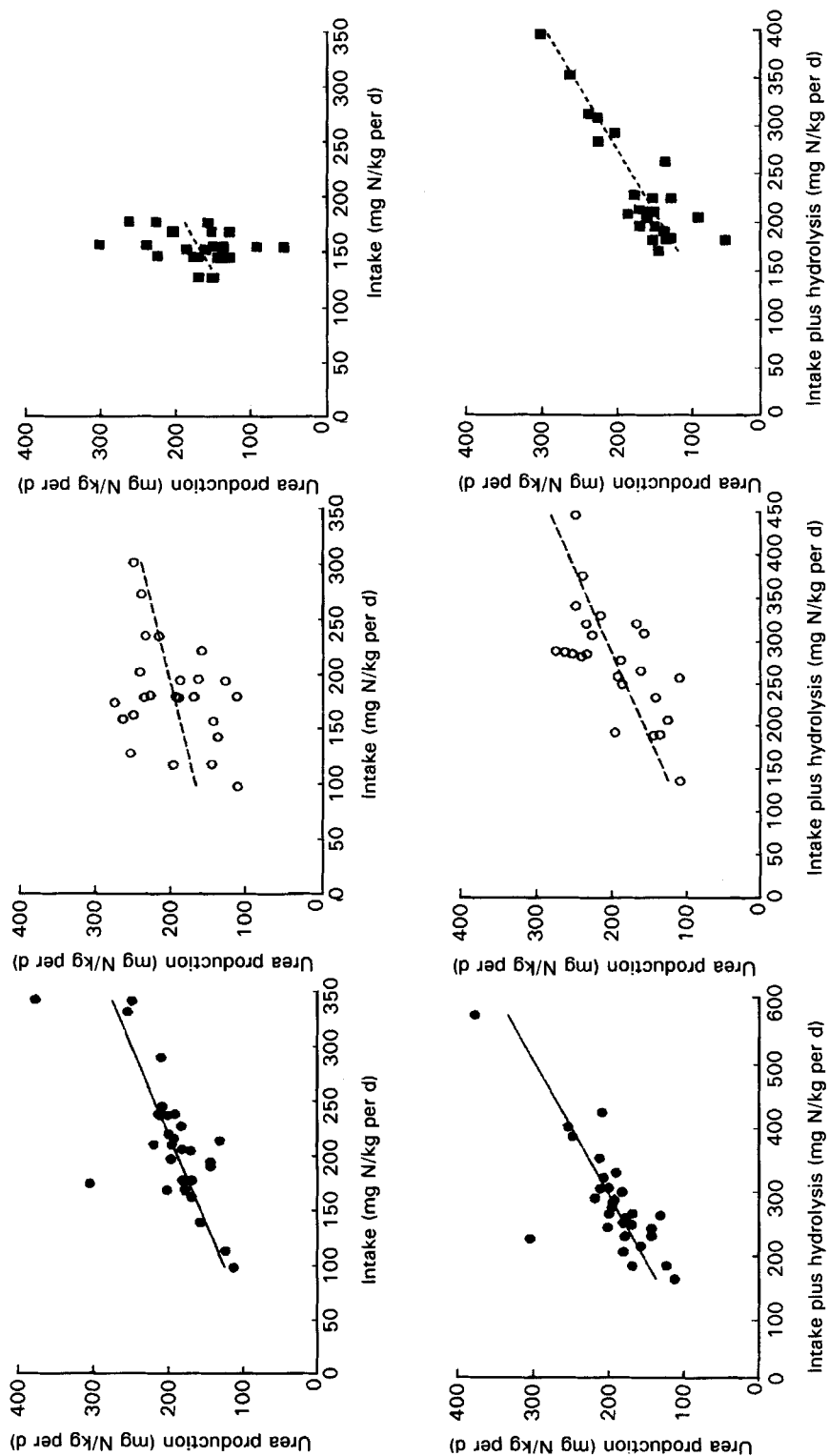


Fig. 2. The rate of urea production was plotted in relation to the dietary intake of nitrogen, and to the dietary intake of N plus urea hydrolysis, at 16, 24 and 32 weeks of gestation for each of three studies in pregnant women: habitual protein intake (HABIT) with primed intermittent oral doses of [¹⁵N]¹⁵N]urea (ID); (●), HABIT with single oral doses of [¹⁵N]¹⁵N]urea (SD); (○) and after 5 d on a diet which provided 60 g protein/d (CONTROL) with SD (□). There were highly significant linear relationships: for HABIT-ID, production v. intake and production v. intake plus hydrolysis (both *P* < 0.0001); for HABIT-SD, production v. intake plus hydrolysis (*P* = 0.0004); for CONTROL-SD, production v. intake plus hydrolysis (*P* < 0.0001). For details of subjects and procedures, see Table 1 and pp. 166–170.

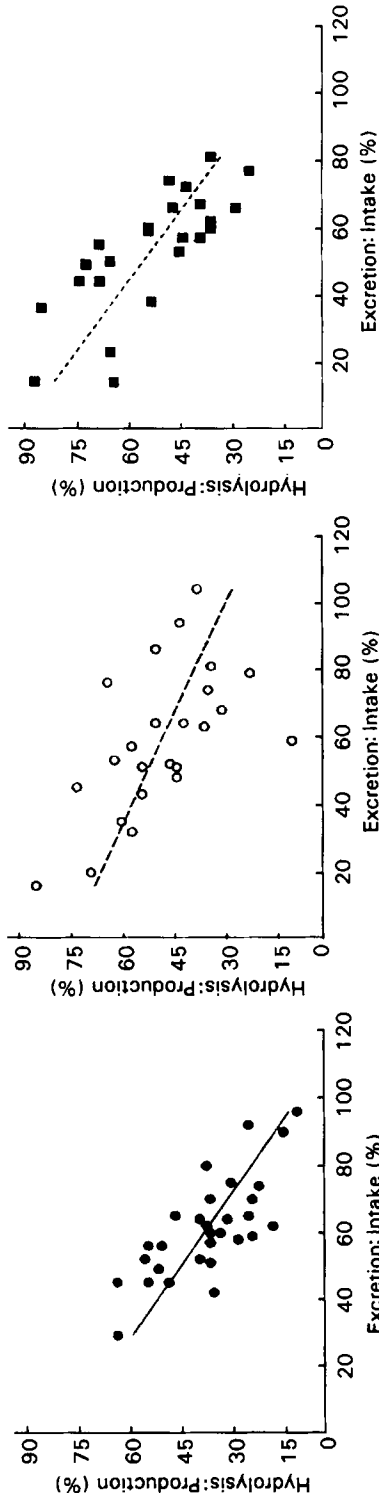


Fig. 3. Urea hydrolysis : urea production was inversely related to urea excretion : nitrogen intake, when urea kinetics were measured at 16, 24 and 32 weeks of gestation for each of three studies in pregnant women: habitual protein intake (HABIT) with primed intermittent doses of [^{15}N] ^{15}N urea: (●; $P < 0.0001$), HABIT with single oral doses of [^{15}N] ^{15}N urea (SD; ○ $P = 0.002$) and after 5 d on a diet which provided 60 g protein/d with SD (□; $P < 0.0001$). For details of subjects and procedures, see Table 1 and pp. 166–170.

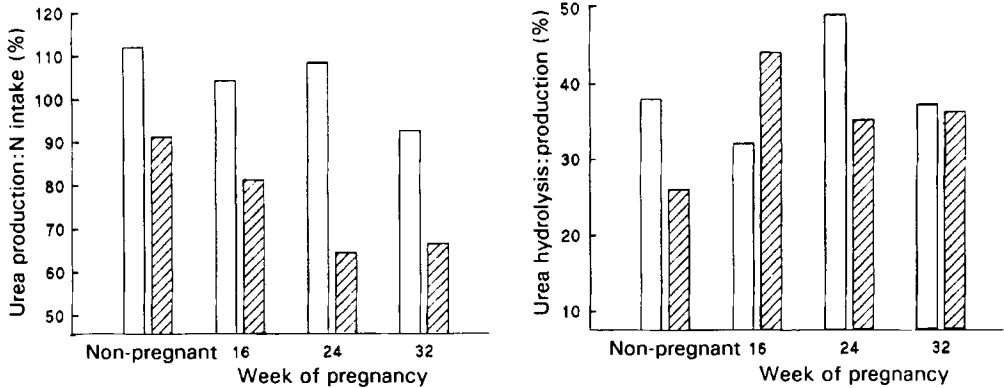


Fig. 4. Urea production, expressed in relation to the dietary intake of nitrogen, and urea hydrolysis, expressed in relation to the rate of urea production, for women in the UK (□), or Jamaica (▨; Forrester *et al.* 1994), taking their habitual protein intake, either in non-pregnant women or during the first, second or third trimesters of pregnancy. For details of UK subjects and procedures, see Table 1 and pp. 166–170.

(Fig. 3). Of the factors which might determine the partitioning of amino acids between protein synthesis and oxidation, the need for amino acids to sustain protein synthesis is quantitatively the most important and is determined by the physiological state. The flow of amino acids to oxidative pathways is presumed to reflect an excess beyond that needed for protein synthesis (Harper *et al.* 1970). This excess may be a consequence of a balanced pattern of amino acids in excess of the need, or a relative mismatch between the pattern of amino acids available compared with the pattern of amino acids needed for the proteins being formed (Harper *et al.* 1970). Either of these options will lead to an increased flow through the oxidative pathway, giving a relatively high value for urea production : N intake. When the need for amino acids is not matched adequately by the intake then there is likely to be increased salvaging of urea-N, with an increase in urea hydrolysis either in absolute terms, or as a proportion of the urea production (Picou & Phillips, 1972; Jackson & Wootton, 1990; Langran *et al.* 1992). On very-low-protein diets there is a lower limit of urea production below which the effective salvaging of urea-N can no longer be achieved, a urea production of approximately 150 mg N/kg per d in normal males (Danielsen & Jackson, 1992; Meakins & Jackson, 1996).

The most marked changes in urea hydrolysis took place during the second trimester, on both the habitual and the controlled protein intake. Maternal weight gain was similar between weeks 16 and 24 and weeks 24 and 32. Therefore, these changes suggest that the pattern of amino acids required at this time might be qualitatively different from that during the earlier stages of pregnancy, because quantitatively absolute fetal growth is greatest in the third trimester. It is difficult to know the exact pattern of amino acids required by the developing fetus, and the demands this might place on the maternal metabolic capability. There is evidence that during the third trimester the requirements for non-essential amino acids might be particularly marked (Widdowson *et al.* 1979; Jackson, 1989). There is an active placento-fetal exchange of amino acids, with the placenta itself engaged in the formation of glycine and glutamine in particular (Lemons & Schreiner, 1984; Christensen, 1992). The fetoplacental exchanges of amino acids appear to be important in satisfying the energy requirements of the fetus, as well as acting as substrates for growth. In the sheep at least 40 % of the energy requirements of the fetus might be derived from amino acid oxidation, with a consequent effect on the rate of urea production

by the fetus (Jackson, 1994). In the human neonate the rate of urea production is also high, about 1.5 g N/kg per d, similar to the rate determined in fetal sheep on a body-weight basis (Steinbrecher *et al.* 1996). It is not known whether the relatively high rate of amino acid oxidation in the neonate is also a reflection of a similar high dependence on amino acid oxidation to satisfy energy requirements to that seen in the fetus. However, if this were the case and the rate of amino acid oxidation in the fetus were similar to neonatal rates then urea formation in the fetus would make an important contribution to the total urea production which has been measured in the mother. For the present study, an estimate of the fetal contribution to urea production would be up to 10 % in the women on their habitual protein intake during mid to late pregnancy, and up to 15 % during late pregnancy in the women on the 60 g protein/d diet.

The results of the present study can be compared with the measurements of urea kinetics made in other studies. For pregnant women in Jamaica (Forrester *et al.* 1994; Fig. 4) in the non-pregnant state and at each stage of pregnancy, there is a lower value for urea production:N intake than for women in the UK, indicative of a greater proportion of the available amino acids being directed towards protein synthesis than to amino acid oxidation. For women in Jamaica, the highest rates for urea hydrolysis:urea production are seen in the first trimester, whereas in the UK the highest rates were in the second trimester. As an isolated observation, this might not seem to have any particular relevance. However, when comparison is drawn between the rates of protein turnover for pregnant women in Jamaica (de Benoist *et al.* 1985) and the UK (Thompson & Halliday, 1992), and the rates of urinary 5-oxoproline excretion in pregnancy between the two locations (Jackson *et al.* 1997), a pattern emerges of differences in the response to pregnancy of protein, amino acid and N metabolism between the two locations. In Jamaican women greatest changes are seen for protein turnover and salvage of urea-N in the first trimester, with increasing 5-L-oxoprolineuria as pregnancy advances, indicative of a poorer nutritional state at the start of pregnancy and less adequate intake during pregnancy. In the UK, the nutritional status of the pregnant women enable the early demands to be accommodated and adaptive responses only become evident in the second trimester as fetal growth increases.

In the two studies where urea production rates were measured only during the third trimester of pregnancy (Kalhan *et al.* 1982; Olufemi *et al.* 1991), the rates were found to be relatively low compared with those of non-pregnant women. In the present study, if the measurements had been restricted to week 32 a similar conclusion might have been drawn. This emphasizes the importance of exploring metabolic changes at each stage of pregnancy and not assuming that the third trimester can be taken to be representative.

The relevance to general metabolism of the salvaging of urea-N is an open debate (Jackson, 1995). The quantities of N which are potentially being made available to metabolism are relatively large, for example during the second trimester the salvaged N was equivalent to approximately 40 g protein/d. The ultimate fate of this N has not yet been clearly defined, but if the N salvaged were used only to retain N and facilitate the formation of non-essential amino acids in the body, this might be of clinical importance for the fetus. We now have direct evidence that some of the N derived from urea might be used for the formation of essential amino acids by the colonic bacteria, and these may be available to the host in amounts which are of functional significance (Jackson, 1995; Yeboah *et al.* 1996).

There is the suggestion that the relationship between fetal growth and the risk of chronic disease, such as diabetes, hypertension and ischaemic heart disease later in life may be directly associated with maternal protein intake during pregnancy (Barker, 1992; Godfrey *et al.* 1996). In animal studies, the programming of metabolic competence similar

to the changes identified in the human condition at risk of chronic diseases can be reproduced by reducing the protein content of the maternal diet to marginal levels (Langley & Jackson, 1994). Therefore, the extent to which a mother is able to make successful adaptations to changes in her dietary protein at different stages of pregnancy, and the consequences for the growth and development of the offspring need to be explored in greater detail.

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