Urea kinetics in healthy young women: minimal effect of stage of menstrual cycle, contraceptive pill and protein intake

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Urea kinetics were measured using prime/intermittent oral doses of [$^{15}N^{15}N$]urea, on five separate protocols in thirteen normal young women. Each woman underwent either two or three study protocols. Measurements were made at day 12 and day 22 of the menstrual cycle, whilst consuming their habitual protein intake in seven women not taking the contraceptive pill and in six women taking the contraceptive pill. In three woman taking the pill, and three not taking the pill, urea kinetics were measured whilst taking a diet in which the intake was restricted to 55 g protein/d. There was no difference in the rate of urea production, urea excretion or urea hydrolysis between the women taking the pill and those not taking the pill at day 22. In the women not taking the pill there was no difference in any measure between day 12 and day 22. In the women taking the pill there was a significant difference in the disposal of urea N to excretion or hydrolysis on day 12 compared with day 22, with a relative decrease in excretion and enhancement of hydrolysis at day 12 compared with day 22. On the restricted diet, an intake of 55 g protein/d represented 77% of the habitual intake and urea production, excretion and hydrolysis were reduced to about 84% of the rate found on the habitual intake. In paired studies the reduction in urea production was statistically significant, and there was a statistically significant linear relationship between urea production and either intake or the sum of intake plus hydrolysis. The within-individual variability for urea production was about 10%, for excretion 15% and for hydrolysis 44%. The between-individual variability for intake was about 17% on the habitual intake. The variability for production, excretion and hydrolysis (14, 13, 36%) was less in the women not taking the contraceptive pill than in those taking the pill (23, 32, 42% respectively). The variability was reduced on the controlled low intake of 55 g protein compared with the habitual intake. These results confirm the wide variability in aspects of urea kinetics between individuals. In women this variability is not, to any large extent, accounted for by changes associated with the menstrual cycle.

Urea: Menstrual cycle: Contraceptive pill: Protein intake

Despite obvious sex differences in metabolism, a significant part of our understanding of energy and nutrient metabolism has been based on studies in men, often young, fit college students who make excellent experimental subjects. It is more difficult to study women, most notably because of the cyclical changes associated with ovulation and menstruation. The studies which have been done in women indicate probable differences in aspects of energy intake, energy expenditure (Bisdee *et al.* 1989) and N intake (Dalvitt-McPhillips, 1983; Gallant *et al.* 1987; Tarasak & Beaton, 1991). Changes in food intake are likely to be associated with changes in N balance (Calloway & Kurzer, 1982; Fong & Kretch, 1993). Not all studies provide information which is concordant, but there is clear evidence of cyclical change, probably of a biphasic nature with an increase in N excretion in the follicular phase followed by a decrease to ovulation, with an increase midway through the

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luteal phase and a further decrease to menstruation. Indicative of intake, and as well as the obvious potential influence of menstrual losses on N metabolism, there is also evidence for more complex modification of intermediary N metabolism throughout the cycle (Calloway & Kurzer, 1982; Gallant *et al.* 1987; Bisdee *et al.* 1989).

Dynamic measures are needed to give a more complete picture of protein status. Protein turnover has been measured in a number of studies (De Benoist *et al.* 1985; Garrel *et al.* 1985; Fitch & King, 1987; Grove & Jackson, 1991). In the studies of Garrel *et al.* (1985) there was some control for the phase of the menstrual cycle and Grove & Jackson (1991) found evidence of an increase in protein turnover premenstrually.

Human subjects can accommodate a wide range of protein intakes and central to the mechanisms involved in this accommodation are aspects of the metabolism of urea (Waterlow, 1968, 1985; Jackson, 1993). There is little information in the literature on urea kinetics in normal, non-pregnant women. Hibbert & Jackson (1991) reported repeated measurements of urea kinetics in a single woman on an adequate intake of N. The day of the cycle was noted, but there was no evidence that this might have contributed to variability in the measurements. Urea kinetics were measured in a group of vegetarian women and there was wide variability between women, which was attributed to the wide differences in protein intake (Bundy *et al.* 1993). As a part of a study on pregnant women, Forrester *et al.* (1994) measured urea kinetics in a group of normal Jamaican women. The results for urea kinetics in women have not in general been different from those for men, but for each group the data show wide inter-individual variation. The basis of the variation is not clear, but in women the stage of menstrual cycle and the use of a contraceptive pill are potentially important.

Since the publication of the report of the expert consultation carried out by the Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU) (1985), which led to the most recent set of international recommendations, there has been heightened controversy over a number of aspects of the recommendations. One of the most active debates surrounds our understanding of the requirements for amino acids and hence protein. We have been interested to note the extent to which the process of the salvaging of urea-N by the metabolic activity of the colonic microflora might make N available to the host, thereby modifying the need for dietary N (Jackson, 1995). This is a complex area and in order for understanding to move forward there is the need to be able to understand the important factors which might contribute to variability in response within and between individuals. In the present paper we have measured urea kinetics in women under different conditions to identify the extent to which the stage of the menstrual cycle, and the use of the contraceptive pill, might contribute to the variability.

METHODS

Subjects and experimental protocols

The studies were carried out in thirteen women aged 21–37 years, with each woman undergoing either two or three study protocols (Table 1). Seven underwent two, and six underwent three protocols. Ethical approval for the studies was given by the joint ethical subcommittee of the Southampton Hospitals and South West Hampshire Health Authority. The women gave consent to their participation after the nature of the investigations had been explained to them. They were all in good health at the time of the studies. The outline of the protocols for each subject is given in Table 1. The timing of all studies was taken from the first day of the last menstrual period. All the women using an oral contraceptive were taking a low-dose oestrogen contraceptive pill.

Table 1. *The age, weight, height and body mass index (BMI) of thirteen young women in whom urea kinetics were measured on two or three different occasions by one of five different protocols**

Subject	Age (years)	Weight (kg)	Height (m)	BMI (kg/m^2)	Protocols
A	25	59·9	1·65	22·1	1, 2, 5
B	24	52·2	1·59	20·6	1, 2, 5
C	23	64·8	1·72	21·9	1, 2, 5
D	21	63·3	1·68	22·4	1, 2
E	24	59·7	1·71	20·2	1, 2
F	25	66·1	1·69	22·9	1, 2
G	37	58·8	1·66	21·3	1, 2
I	25	54·2	1·65	20·0	3, 4, 5
J	29	61·7	1·65	22·5	3, 4, 5
K	23	54·6	1·65	20·0	3, 4, 5
L	24	79·6	1·60	31·1	3, 4
M	21	56·6	1·70	19·6	3, 4
N	22	64·2	1·71	21·9	3, 4

* For details of protocols, see p. 201.

Protocol 1. In seven women (A–G) urea kinetics were measured whilst they were taking their habitual diet. None of the women was using a contraceptive pill and the studies were carried out on day 12 of the cycle.

Protocol 2. In seven women (A–G) urea kinetics were measured whilst they were taking their habitual diet. None of the women was using a contraceptive pill and the studies were carried out on day 22 of the cycle.

Protocol 3. In six women (I–N) urea kinetics were measured whilst they were taking their habitual diet. All of the women were using a contraceptive pill and the studies were carried out on day 12 of the cycle.

Protocol 4. In six women (I–N) urea kinetics were measured whilst they were taking their habitual diet. All of the women were using a contraceptive pill and the studies were carried out on day 22 of the cycle.

Protocol 5. In six women (A–C, I–K) urea kinetics were measured whilst they were taking a diet which provided 55 g protein/d. Three of the women were taking the contraceptive pill at the time of the study. There was no control for the day of the cycle on which the study was carried out.

Diets

For each protocol the diet was derived from ordinary foodstuffs and was provided in the form of five isonitrogenous meals of similar energy content each day. The food was prepared and delivered to the subjects' homes. The diets for all groups were composed of similar types of foods and designed as far as possible to mimic habitual eating patterns. The 06.00 and 09.00 hours meals consisted of breakfast cereal, fruit juice and toasted bread. At 12.00 and 15.00 hours sandwiches, biscuits and fruit juice were provided. A ready-prepared meal from a reputable commercial outlet was provided at 18.00 hours and was usually based on pasta taken with a salad. Drinks were provided to suit individual taste. Flexibility in the choice of menu items was allowed to take account of individual preferences. Measures of food intake were coded and analysed for energy and protein content using a computerized database (Microdiet, University of Salford, Greater Manchester). Similar foods were eaten by all the women and there were no obvious differences in intake in

relation to the contraceptive pill. The NSP intake between the groups was similar, although this was not tested formally.

In Protocols 1–4 an estimation of habitual dietary intake was determined on the basis of a 3 d weighed food record which comprised two week days and one weekend day. Each subject received an intake of energy and protein similar to their habitual intake. In Protocol 5 an estimation of habitual dietary intake was determined based on a 24 h recall. Each subject received her habitual intake of energy, but the protein content of the diet was restricted to 55 g/d, the recommended daily allowance (RDA) for the UK at the time (Department of Health and Social Security, 1979).

Measurement of urea kinetics

Each study protocol lasted for a 2 d period with urea kinetics being measured using the prime/intermittent oral presentation of [$^{15}N^{15}N$]urea, with collections of urine during the final 24 h of the period. At 21.00 hours on the first day of the study the subject emptied her bladder. A specimen of urine was collected for the measurement of baseline enrichment. After 3 h, at midnight, a priming dose of [$^{15}N^{15}N$]urea, 28·5 mg (99% atoms ^{15}N, Cambridge Isotope Laboratories, MA, USA), equivalent to 15 h of intermittent infusion 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 [$^{15}N^{15}N$]urea, 5·5 mg were administered at intervals of 3 h until 15.00 hours. Urine was collected immediately before the administration of the first dose of isotope and at intervals of 3 h from 06.00 until 21.00 hours. An accurately known amount of [$^{15}N^{15}N$]urea was made up in sterile water and kept on ice until ready for use.

Analyses

All specimens of urine were collected into acidified containers and stored frozen until later analysis. 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), and hence the relative proportions of [$^{15}N^{15}N$]urea, [$^{15}N^{14}N$]urea and [$^{14}N^{14}N$]urea can be determined. Measurements were carried out in a triple collector isotope-ratio mass spectrometer (SIRA 10, VG Isogas, Winsford, Ches.).

Calculations

Following the prime/intermittent presentation of labelled [$^{15}N^{15}N$]urea an isotopic steady state is achieved in urinary urea. At this point the dilution of label gives a measure of the rate at which urea is being produced endogenously in the body (Pu). The rate of urea excretion in urine can be measured directly (Eu), and from this the rate at which urea is being hydrolysed (T) by the colonic microflora and the urea-N salvaged for further metabolic interaction can be calculated ($T = Pu - Eu$) (Jackson *et al.* 1984).

Comparisons between groups of data were carried out using the paired t test or ANOVA, and differences were considered to be statistically significant for a value of $P < 0{\cdot}05$. Results are reported as the mean and the standard deviation.

RESULTS

All the studies were completed satisfactorily. A total of thirty-two measurements of urea kinetics were made in thirteen women with six of the women having measurements made on three separate occasions. The women ingested the diets or kept the weighed records as planned and successfully collected urine samples for the time periods specified.

Plateau enrichment in urinary urea was identified by visual inspection with a satisfactory plateau achieved in all the studies. In protocols 1 and 2 and protocols 3 and 4 a comparison was made for the effect of stage of menstrual cycle, as the only difference in the study design was the day of cycle on which the measurements were made. A comparison for the effect of the use of a contraceptive pill was possible between protocols 1 and 3, and protocols 2 and 4, as the women were selected according to their usage of a contraceptive pill. A comparison for the effect of intake of protein was possible between protocol 5 and protocols 1, 2, 3 and 4, as the protein content of the diet in protocol 5 was lower compared with the habitual diet taken in protocols 1, 2, 3 and 4.

Table 2 shows the urea kinetics for each individual for each of the studies on day 12 and day 22 of the cycle on the habitual intake, and on a restricted intake of 55 g protein. The CV for N intake, urea production and excretion was 17–19%, and for urea hydrolysis 29% (see Table 5). Of the N derived from urea hydrolysis, 26% was returned to urea formation and 74% retained within the metabolic N pool. The CV for intake plus hydrolysis was 3%.

Table 3 shows a comparison of urea kinetics between day 12 and day 22 of the menstrual cycle between the group not taking the contraceptive pill and those taking the contraceptive pill on a regular basis. For the group as a whole there were no differences in any aspect of urea kinetics between day 12 and day 22. Similarly there were no differences between day 12 and day 22 in the group who were not taking the contraceptive pill. In the group taking the contraceptive pill, however, there was a significant difference in the disposal of urea N between day 12 and day 22. In this group of women a significantly greater proportion of the urea produced was retained on day 12 than on day 22 of the cycle. As a group, the women on the contraceptive pill tended to have a lower protein intake, but this difference did not reach statistical significance. Most aspects of urea kinetics were similar between the two groups, other than the relative disposal of urea N in the women on the contraceptive pill on day 12 of the cycle who hydrolysed a greater proportion of urea production than any other group (47% of production, compared with 32–38% of production). There was a close relationship between urea production and the sum of urea intake and hydrolysis, so that on average production was about 78–80% of intake plus hydrolysis (Fig. 1).

In Table 4 a comparison is drawn between the urea kinetics on an habitual intake of dietary protein and those when the dietary protein was restricted to 55 g/d. The subgroup of six who were studied on the controlled, lower protein intake tended to have higher habitual intakes than the total group from which they were drawn. There were no differences in urea kinetics in the subgroup between days 12 and 22 of the cycle. The 55 g protein diet contained about 77% of the protein taken habitually. On this diet, urea production was decreased to about 84% of that seen on the habitual diet, as was urea excretion and urea hydrolysis. Although these differences did not reach statistical significance when compared with the group as a whole, there were significant differences when paired comparisons were made for the subjects on the 55 g protein diet. Compared with the restricted group, urea production on day 12 just failed to reach a conventional level of statistical significance ($P = 0{\cdot}052$), but did for day 22 ($P = 0{\cdot}015$), as did the mean for days 12 and 22 ($P = 0{\cdot}012$). When urea production in the restricted group was compared with all the values on day 12 and day 22, for the same subjects in an unpaired test, there was a highly statistically significant difference ($P = 0{\cdot}0084$). There was a strong linear correlation between N intake and urea production for all the measurements, Fig. 1 (r 0·695, $P = 9{\cdot}98 \times 10^{-6}$). The relationship was even stronger between the sum of N intake plus hydrolysis and urea production (r 0·83, $P = 4{\cdot}31 \times 10^{-9}$).

Table 5 summarizes the variability in the measurements of urea kinetics within individuals. On habitual diets the variability in the protein intake between the study on day 12 and day 22 was low, less than 2% overall. The variability in urea production was on

Table 2. *Urea kinetics, measured in thirteen young women on two occasions at day 12 and day 22 of the menstrual cycle, while they were taking their habitual intake of protein and energy and again in six women while taking a restricted protein intake which provided 55 g protein/d**

Subject	Study	Intake (mg N/kg per d)	Production (mg N/kg per d)	Excretion (mg N/kg per d)	Hydrolysis (mg N/kg per d)	Recycled to urea formation (mg N/kg per d)	Intake plus hydrolysis (mg N/kg per d)
A	Habitual intake, d 12	244	195	142	52	20	296
	Habitual intake, d 22	247	245	130	115	17	362
	55 g protein	146	208	153	54	20	201
B	Habitual intake, d 12	240	242	149	93	30	333
	Habitual intake, d 22	238	255	159	95	30	334
	55 g protein	178	183	107	75	27	254
C	Habitual intake, d 12	164	180	146	34	14	198
	Habitual intake, d 22	163	199	142	57	28	220
	55 g protein	141	175	77	98	20	239
D	Habitual intake, d 12	159	174	103	70	12	229
	Habitual intake, d 22	159	191	125	66	15	225
E	Habitual intake, d 12	197	228	128	100	18	297
	Habitual intake, d 22	192	202	152	50	18	242
F	Habitual intake, d 12	154	149	108	42	12	195
	Habitual intake, d 22	151	189	122	67	14	218
G	Habitual intake, d 12	181	194	150	44	20	225
	Habitual intake, d 22	181	188	116	71	25	252
I	Habitual intake, d 12	160	217	134	83	24	243
	Habitual intake, d 22	160	212	122	90	24	250
	55 g protein	152	187	124	63	17	216
J	Habitual intake, d 12	188	244	105	139	36	327
	Habitual intake, d 22	186	187	154	32	16	219
	55 g protein	144	183	127	56	16	199
K	Habitual intake, d 12	193	255	132	123	22	316
	Habitual intake, d 22	193	218	88	130	17	322
	55 g protein	159	179	79	100	24	259
L	Habitual intake, d 12	121	125	47	78	12	199
	Habitual intake, d 22	120	140	77	63	11	183
M	Habitual intake, d 12	169	149	109	40	14	209
	Habitual intake, d 22	169	164	98	67	14	235
N	Habitual intake, d 12	183	234	113	122	31	304
	Habitual intake, d 22	183	237	177	60	36	243

* For details of subjects, diets and procedures, see Table 1 and pp. 200–202.

Table 3. *Urea kinetics measured in thirteen women (six taking the oral contraceptive pill, and seven not taking the oral contraceptive pill) whilst taking their habitual diets on either day 12 or day 22 of the menstrual cycle**

(Mean values and standard deviations)

	Total (*n* 13)				Without contraceptive pill (*n* 7)				With contraceptive pill (*n* 6)			
	Day 12		Day 22		Day 12		Day 22		Day 12		Day 22	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Intake (mg N/kg per d)	181	34	180	34	191	38	190	38	169	26	168	27
Production (mg N/kg per d)	199	42	202	32	195	32	210	28	204	54	193	36
Excretion (mg N/kg per d)	121	28	128	29	132	20	135	16	107	32	119	39
Hydrolysis (mg N/kg per d)	78	35	74	27	62	26	75	23	97	37	74	33
Production/intake (%)	111	17	113	12	103	12	112	10	120	19	115	15
Excretion/production (%)	61	14	63	11	69	10	65	7	53	13	62	15
Intake plus hydrolysis (mg N/kg per d)	259	53	254	52	253	54	265	59	266	57	242	46
Production/intake plus hydrolysis (%)	77	8	80	9	78	9	80	11	76	9	80	11

* For details of subjects, diets and procedures, see Table 1 and pp. 200–202.

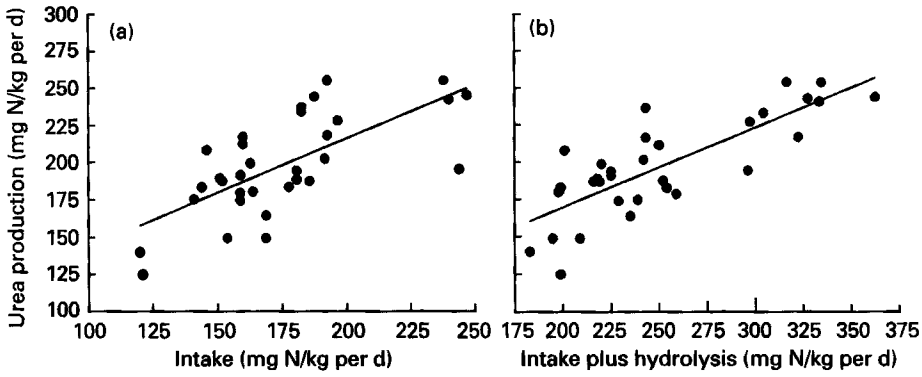

Fig. 1. Urea kinetics were measured in thirteen women on both day 12 and day 22 of the menstrual cycle whilst taking their habitual dietary protein and in six of these whilst taking a restricted intake of 55 g protein/d. The rate of urea production for each study period was related to both the dietary intake of nitrogen (a), and to the sum of dietary nitrogen intake and the hydrolysis of urea by the colonic microflora (b). By linear regression analysis urea production was more closely related to the sum of intake and hydrolysis (r 0·83, $P = 4{\cdot}3 \times 10^{-9}$) than to intake alone ($r$ 0·70, $P = 9{\cdot}98 \times 10^{-6}$).

Table 4. *Urea kinetics in a group of six women taking a controlled intake of 55 g protein/d, compared with urea kinetics whilst taking their habitual intake with measurements being made on either day 12 or day 22 of the menstrual cycle**

(Mean values and standard deviations)

	Habitual intake, total (n 13)		Habitual intake, subgroup day 12 (n 6)		Habitual intake, subgroup day 22 (n 6)		Controlled intake, subgroup (n 6)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Intake (mg N/kg per d)	181	34	198	31	198	37	153	14
Production (mg N/kg per d)	200	34	222	30	219	26	186	12
Excretion (mg N/kg per d)	124	24	135	16	133	26	111	30
Hydrolysis (mg N/kg per d)	76	22	87	40	87	36	74	21
Intake plus hydrolysis (mg N/kg per d)	257	48	285	54	284	63	228	26
Production/intake (%)	112	13	115	22	112	13	122	13
Excretion/production (%)	62	8	61	15	61	15	59	13
Production/intake plus hydrolysis (%)	79	7	79	10	79	10	83	14

* For details of subjects, diets and procedures, see Table 1 and pp. 200–202.

average 8% (range 0·8–19%). There was much wider variability in urea excretion (mean 16%, range 2–32%) and urea hydrolysis (mean 30%, range 2–88%), and for each the variability was greater in the subjects taking the contraceptive pill than those not on the pill. In protocols 1 and 2 the variability between individuals for intake was 20%, and 16% for protocols 3 and 4. For protocols 1 and 2 the variability in urea production and excretion was about 12–16%, and for urea hydrolysis about 35%. In protocols 3 and 4 the variability was greater, for urea excretion about 23% and for urea hydrolysis about 42%. Controlling the intake of protein reduced the variability in intake to 9% and in production to only 6%, with excretion and hydrolysis being about 30% and 25% respectively.

Table 5. *The within-subject variability in measures of urea kinetics in thirteen young women in whom measurements were taken on both day 12 and day 22 of the menstrual cycle, expressed as coefficient of variation* (%)

Subject	Protocol	Intake	Urea production	Urea excretion	Urea hydrolysis
A	1, 2	0·95	16·0	32·3	52·8
B	1, 2	0·53	3·6	4·4	2·3
C	1, 2	0.43	7·1	2·0	35·6
D	1, 2	0·08	7·0	13·0	4·0
E	1, 2	2·0	9·0	12·0	47·0
F	1, 2	1·0	17·0	9·0	33·0
G	1, 2	0·08	2·0	18·0	34·0
I	3, 4	0	1·7	6·5	5·5
J	3, 4	0·72	18·9	26·8	88·0
K	3, 4	0	11·3	28·4	3·9
L	3, 4	0·7	8·0	34·0	15·0
M	3, 4	0	7·0	8·0	35·0
N	3, 4	0	0·84	31·0	47·0
Range		0–0·95	0·84–18·9	2·0–34·0	2·3–88·0

DISCUSSION

This series of studies was designed to measure urea kinetics in healthy young women, to establish if values were similar to those of men, to consider some of the factors which may contribute to variation in the measurements and to quantify the extent of this variation between and within the women studied. As a point of reference, urea kinetics were measured when the women were receiving 55 g protein, equivalent to the RDA at the time, which represented a 23% reduction in their habitual protein intake.

The results show that in free-living women, taking their habitual intake of protein, there was little effect on urea kinetics of the time of the menstrual cycle at which the measurements were made. In general there was very little difference between women who were taking the contraceptive pill compared with those who were not, except that in the women taking the contraceptive pill there was decreased excretion and enhanced hydrolysis of urea at about day 12 of the cycle. When the protein intake of the women was restricted to 55 g/d (about 77% of habitual), there was a less marked reduction in urea production, excretion and hydrolysis (about 84% of that on the habitual diet). In paired comparisons there was a significant reduction in urea production on 55 g protein/d compared with the habitual intake, for both day 12 and day 22. There appeared to be a progressive fall in production with intake over the range of protein intakes studied (Fig. 1). The results for urea kinetics (expressed as mg N/kg per d) were similar to those obtained for non-pregnant Jamaican women by Forrester *et al.* (1994), and by Hibbert & Jackson (1991) (Table 6). The results in women were also similar to those found in men in the UK (expressed as mg N/kg per d) (Langran *et al.* 1992; Danielsen & Jackson, 1992).

For each protocol in the present study, urinary urea was approximately 70% of N intake, and urea production was 100–120% of intake.

The values obtained for within-individual variation and between-individual variation were similar to those reported by Hibbert & Jackson (1991) for repeat measurements in one woman and considerably less than the variability reported by Walser & Bodenlos (1959) in a group of men. The least variation amongst individuals was seen for urea production during protocol 5, where the intake of protein was controlled at 55 g protein/d. Even here,

Table 6. *A comparison of the values for urea kinetics obtained in the present study on normal women with those in the literature in which a similar method has been used in normal adults of either sex*

Reference		Intake	Production	Excretion	Hydrolysis	Production/ intake	Excretion/ production
Present study	Female	181	200	124	76	112	62
Present study, low protein	Female	153	186	111	74	122	59
Forrester *et al.* (1994)	Female	167	150	110	40	91	66
Hibbert & Jackson (1991)	Female	231	198	143	55	86	72
Danielsen & Jackson (1992)	Male	165	199	118	80	121	60
Langran *et al.* (1992)	Male	149	194	101	92	132	54

there was much greater variability in urea excretion and urea hydrolysis. Overall, the variability in urea production is much less than would have been expected from urea excretion, within individuals, between individuals, and across different levels of dietary protein intake. The measurement of urea production is based directly on the dilution of the dose of labelled [$^{15}N^{15}N$]urea by the urea being produced endogenously and in principle, therefore, it is the measure in which one has most confidence. The variation in plateau enrichment for [$^{15}N^{15}N$]urea was 11·8 (SD 6·5)% for all the studies. Taken together these results suggest that there are important factors which determine the balance of the rate of urea excretion and hydrolysis, within and between individuals, which have not been considered so far.

When the protein intake was restricted to 55 g/d, in protocol 5, there was a modest but statistically significant reduction in urea kinetics, of similar magnitude to the change seen in men when protein intake was progressively decreased (Danielsen & Jackson, 1992; Langran *et al.* 1992). However, in this group of women there was no associated increase in hydrolysis as the intake of protein was reduced. No time was allowed for equilibration on the lower protein diet, and this might account for the failure to measure a change in hydrolysis. In reality the habitual protein intake for some women, as assessed by the 24 h dietary recall, was considerably more than the RDA, 55 g/d.

The other statistically significant difference of note in the present study was in the women who were taking the contraceptive pill, for whom there was a relative decrease in the excretion of urea on day 12 of the cycle. We are not able to state at this time whether this difference is of biological importance. The available information on how female hormones affect N retention and excretion in women is limited and often contradictory. These studies have shown that healthy young women have similar urea kinetics values to those of men. In paired studies there was an effect of the level of protein intake. Whereas in women not taking a contraceptive pill there was no measurable effect of the menstrual cycle, in women taking the contraceptive pill there was evidence of altered urea kinetics during the pre-ovulatory phase, which is deserving of further investigation. The basis of the wide interindividual variability in the relative excretion and salvage of urea N is not clear. The differences are greater than can be accounted for by measurement error and are suggestive of genuine biological variation.

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