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Nitrogen balance and d15N: why you're not what you eat during pregnancy

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RCM

Nitrogen balance and δ^{15}N: why you're not what you eat during pregnancy

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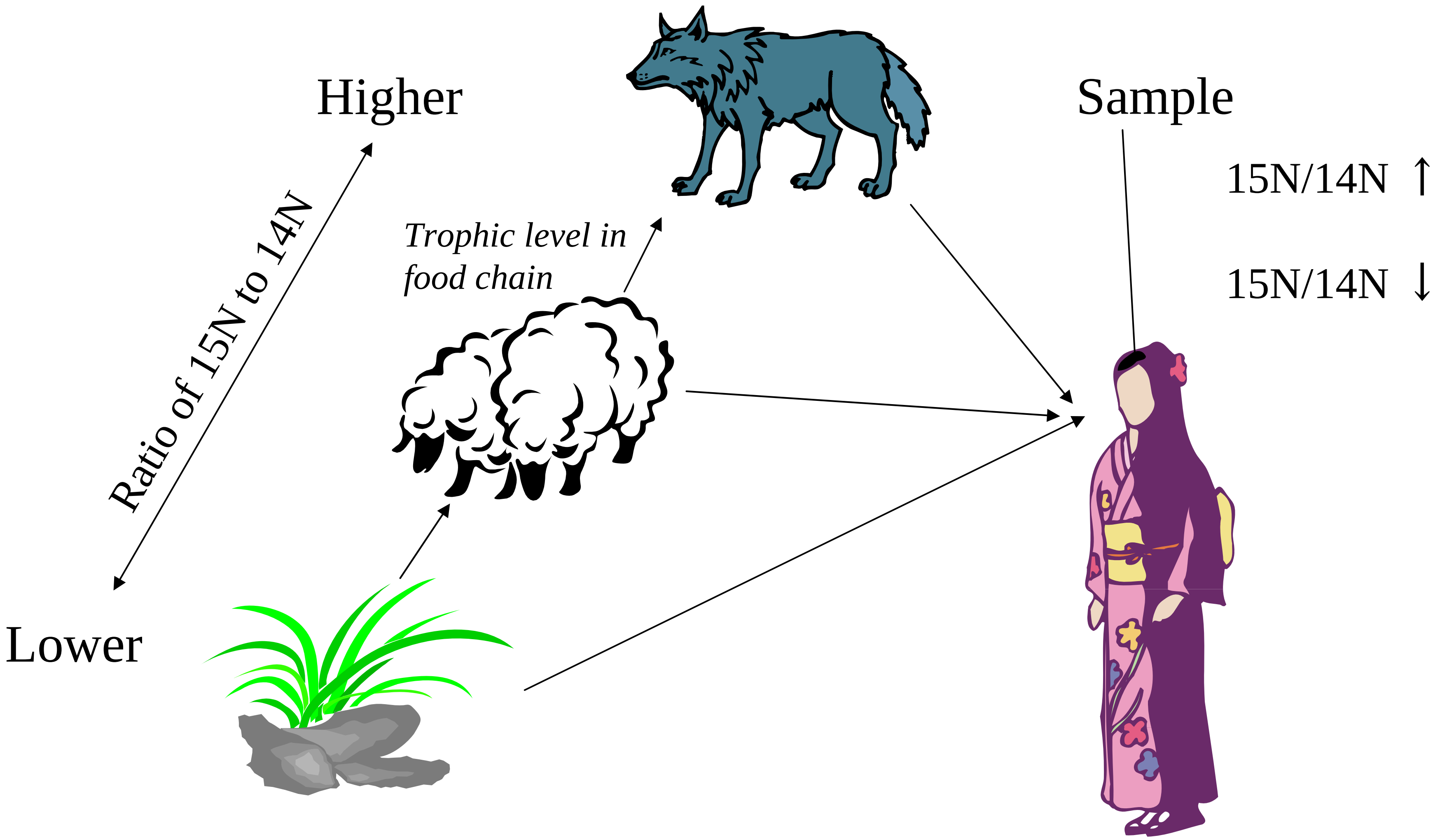
Carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) stable isotope ratios were longitudinally measured in human hair that reflected the period from pre-conception to delivery in 10 pregnant women. There was no significant change in the $\delta^{13}C$ results, but all subjects showed a decrease in $\delta^{15}N$ values (−0.3 to −1.1‰) during gestation. The mechanisms causing this decrease in hair $\delta^{15}N$ have not been fully elucidated. However, since the $\delta^{15}N$ values of dietary nitrogen and urea nitrogen are significantly lower compared to maternal tissues, it is hypothesized that the increased utilization of dietary and urea nitrogen for tissue synthesis during pregnancy resulted in a reduction of the steady state diet to a body trophic level effect by approximately 0.5–1‰. An inverse correlation ($R^2 = 0.67$) between hair $\delta^{15}N$ and weight gain was also found, suggesting that positive nitrogen balance results in a reduction of $\delta^{15}N$ values independent of diet. These results indicate that $\delta^{15}N$ measurements have the ability to monitor not only dietary inputs, but also the nitrogen balance of an organism. A potential application of this technique is the detection of fertility patterns in modern and ancient species that have tissues that linearly record stable isotope ratios through time.

Background (I)

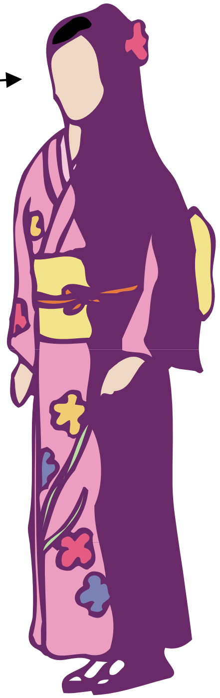
Nitrogen: stable isotopes 15 N (0.365%), 14N (99.635%)



Background (I)

Enrichment factor or
Fractionation factor = (body d15N) / (dietary d15N) = 3-4‰

Dietary d15N



d15N in body
protein pool

Fractionation factor is
stable for sex and age

Background (I)

$\delta\,^{15}N$ =

$$d15N = \{(15N/14N_{(sample)} - 15N/14N_{\underline{(standard)}}) / 15N/14N_{(standard)}\} \times 1000$$

Atomospheric N

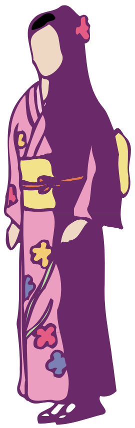
$\delta\,^{13}C$ =

$$d13C = \{(13C/12C_{(sample)} - 13C/12C_{\underline{(standard)}}) / 13C/12C_{(standard)}\} \times 1000$$

Fossil remain of shell

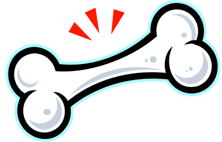
d13C: higher in C3 plants (e.g., rice, wheat, potato)

lower in C4 plants (e.g., maize)



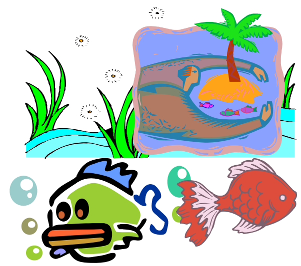
Background (I)

In archaeology,



d15N
d13C → Diet in the past

In ecology,



d15N
d13C → Analysis of food web in the regional ecosystem

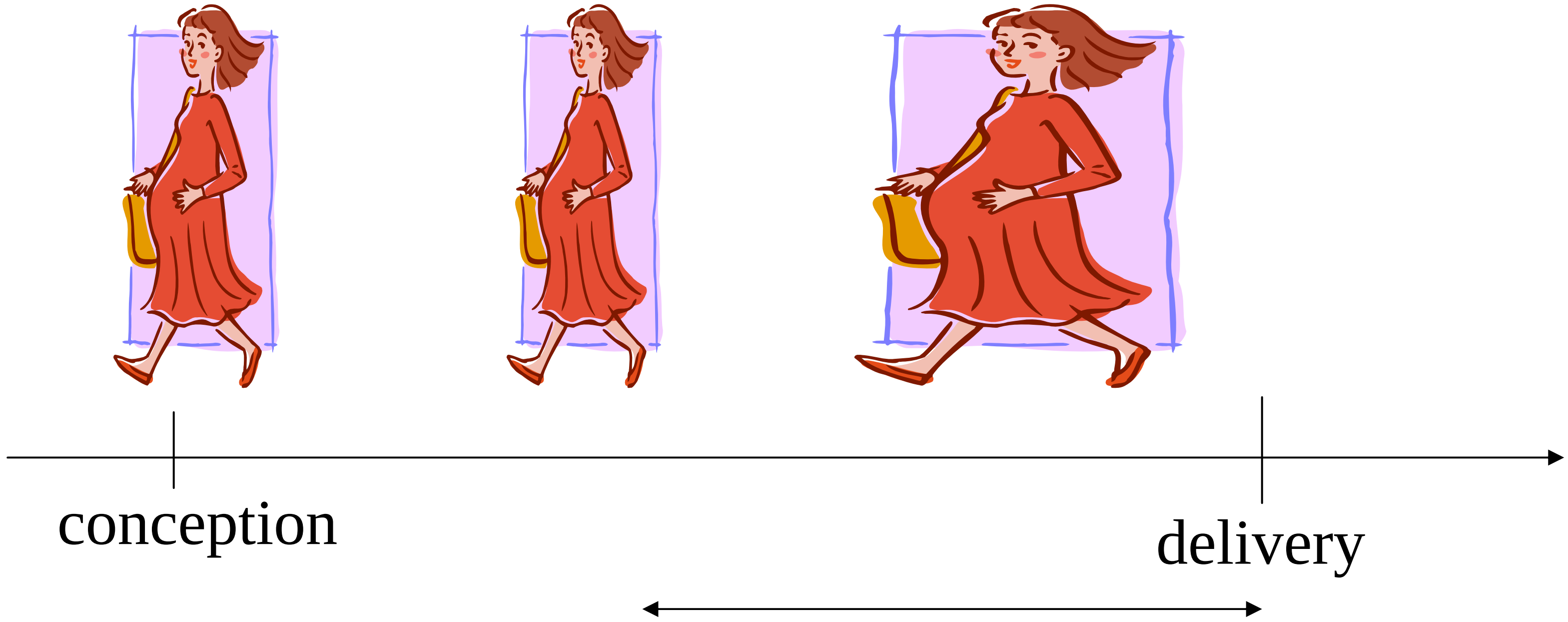
Preconditions: (1) "You are what you eat"
(2) Fractionation factor is stable

The principle is valid for a steady metabolic state.

Recent study: <u>Negative nitrogen balance (nutritional stress, diseases) increase d15N.</u> Positive nitrogen balance??

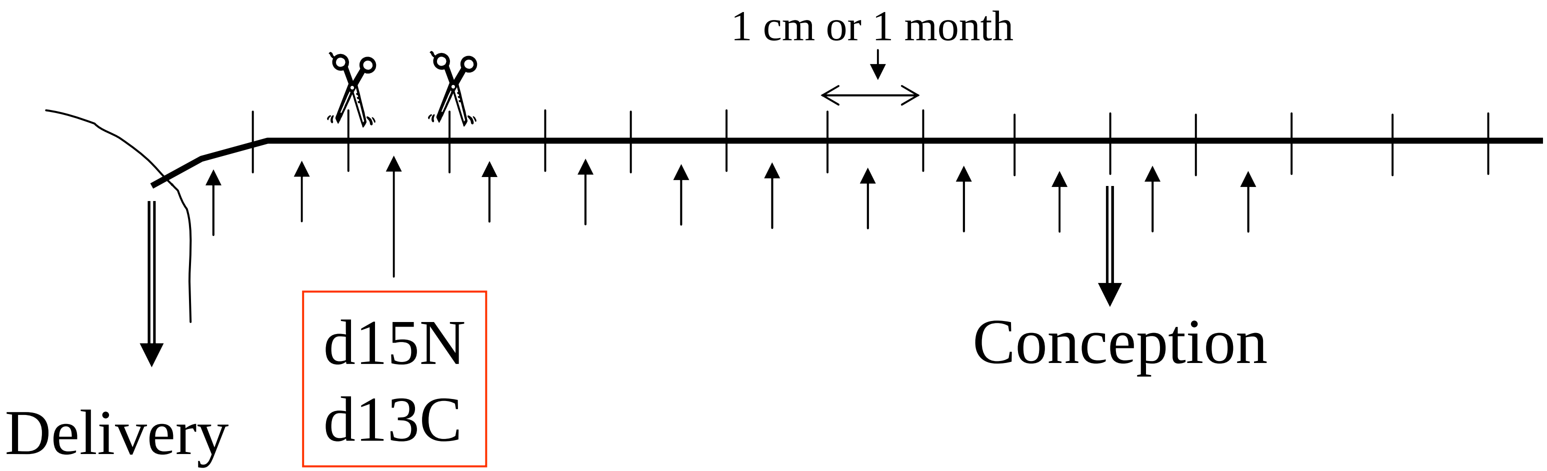
d15N change under positive nitrogen balance

Longitudinal change of d15N/d13C throughout human pregnancy



Subjects: 10 women

Sample: hair just after the delivery



Dietary survey: every 6-8 weeks (EPIC FFQ)

Body weight: every months (continuously?)

The Result

No systematic changes in the diets of the individuals during gestation.

Table 1. Hair $\delta^{13}C$ and $\delta^{15}N$ values at conception and birth for subjects A–J. Also included are the infant birth weights

Subject	$\delta^{13}C$ at conception (‰)	$\delta^{13}C$ at birth (‰)	$\delta^{15}N$ at conception (‰)	$\delta^{15}N$ at birth (‰)	Birth weight (kg)
A	−17.3	−17.7	8.9	7.8	3.78
B	−16.9	−17.1	9.1	8.7	2.85
C	−16.8	−17.0	8.9	7.9	3.95
D	−17.8	−17.9	8.9	8.0	4.90†
E	−17.6	−17.2	8.7	7.9	3.50
F	−16.9	−16.7	9.3	8.5	3.82
G	−18.0	−17.5	8.9	8.3	3.86
H	−18.1	−17.7	8.6	7.9	3.38
I	−18.0	−18.2	9.3	9.0	3.63
J 1st*	−17.1	−17.2	9.4	8.9	3.05
J 2nd*	−17.1	−16.9	9.2	8.9	2.92

*Subject J's hair $\delta^{15}N$ values recorded two successive pregnancies.
† Twins

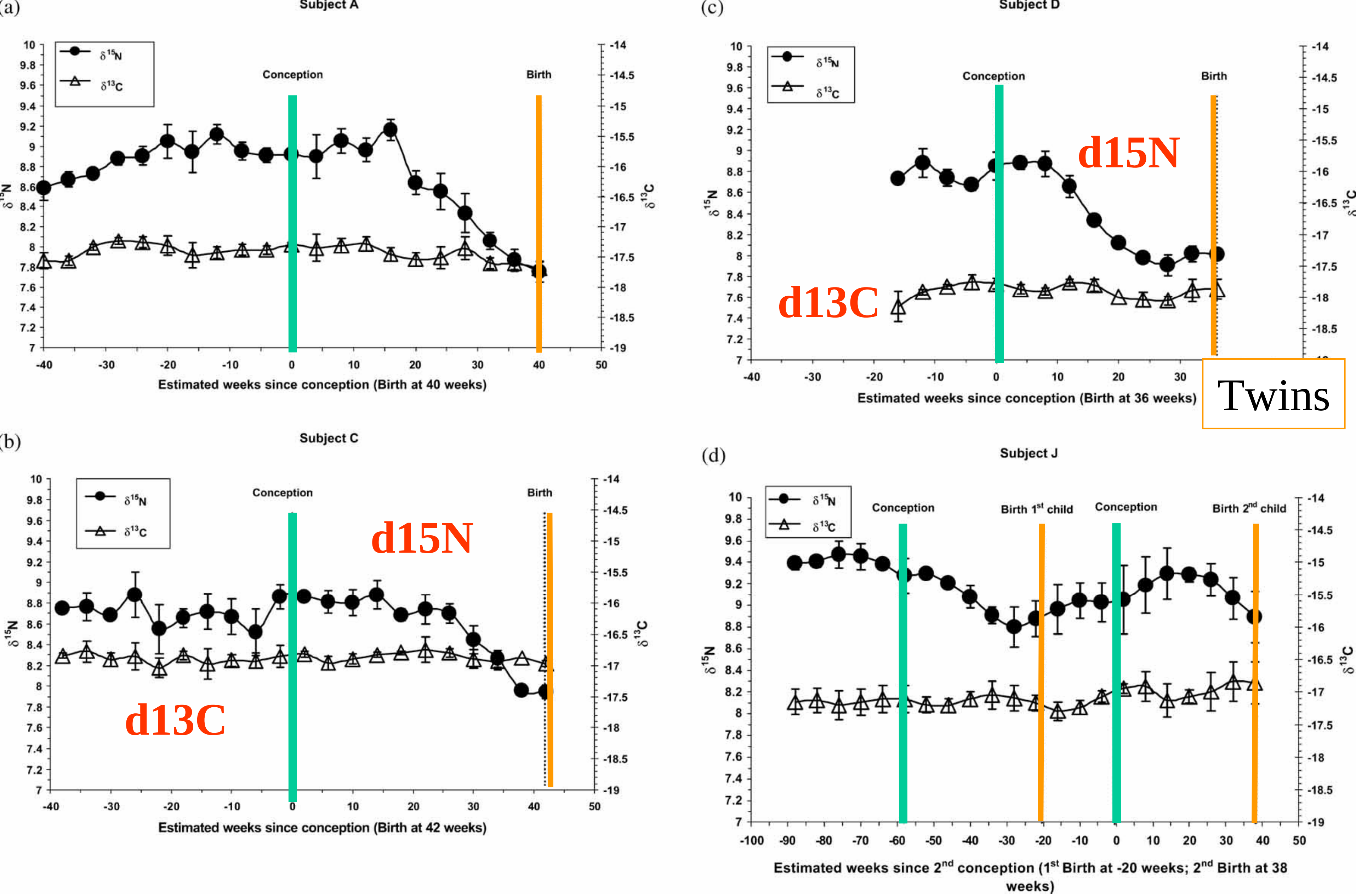


Figure 2. Representative graphs of typical *d¹³C and d¹⁵N variations before and during pregnancy in human hair sampled at birth (a, b). Hair samples were analyzed in 1 or 1.5 cm sections corresponding to 4 or 6 week intervals of growth, respectively,*[38] *and thus the x-axis is time derived from measurement along the hair starting from the scalp. All samples were measured in triplicate with the error bars shown. In (c), subject D gave birth to twins, and in (d) the hair of subject J was sufficiently long to record two successive pregnancies.*

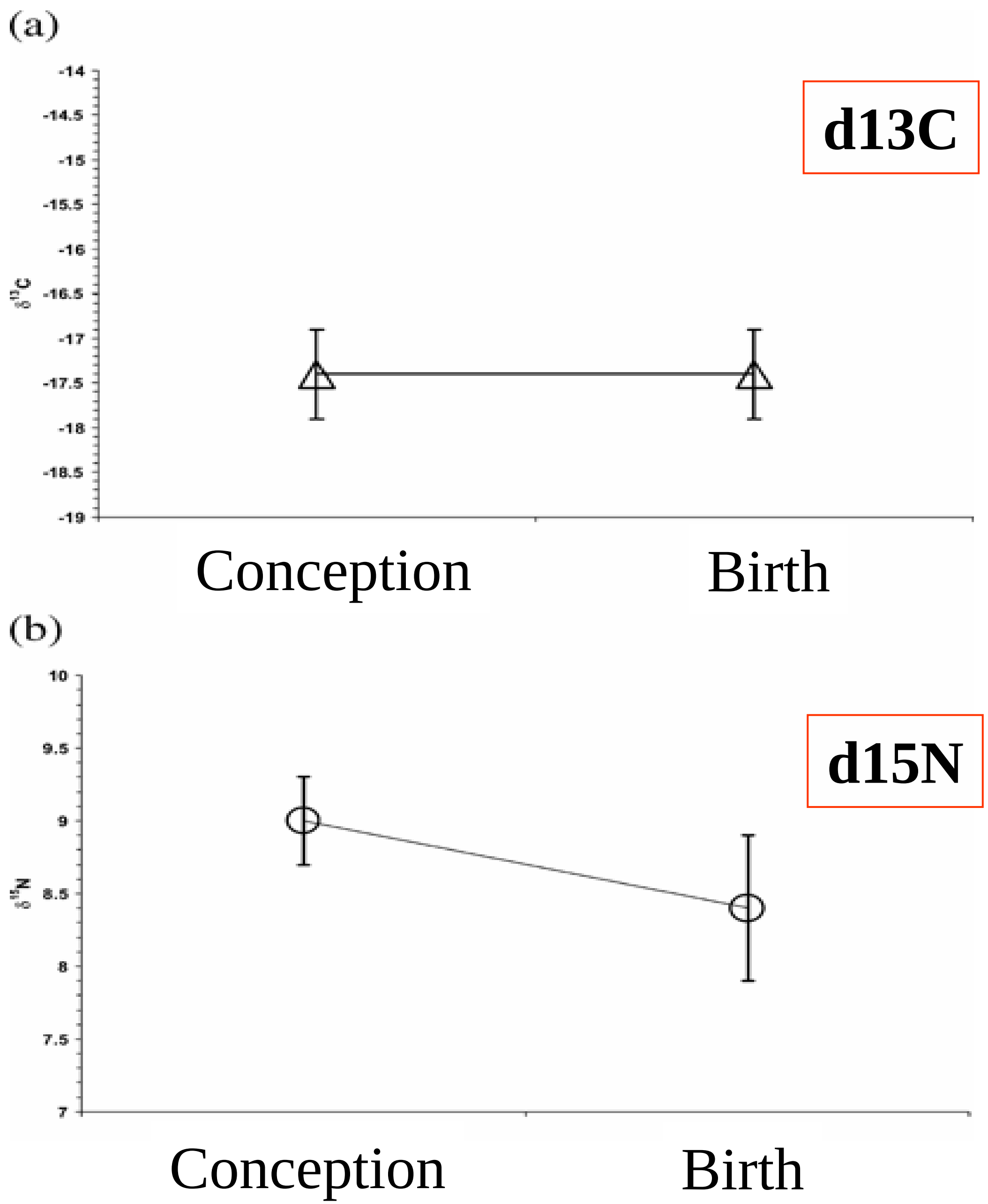


Figure 1. Graphs illustrating the change between conception and birth (mean, SD) for the hair *d*13C (a) and *d*15N (b) results from 10 pregnant women. There is no consistent variation in the *d*13C values (conception = -17.4, 0.5 ; birth = -17.4, 0.5), but all subjects show a significant decrease in *d*15N between conception (9.0, 0.3) and birth (8.4, 0.5).

Weight gain =protein (8%) + fat + water

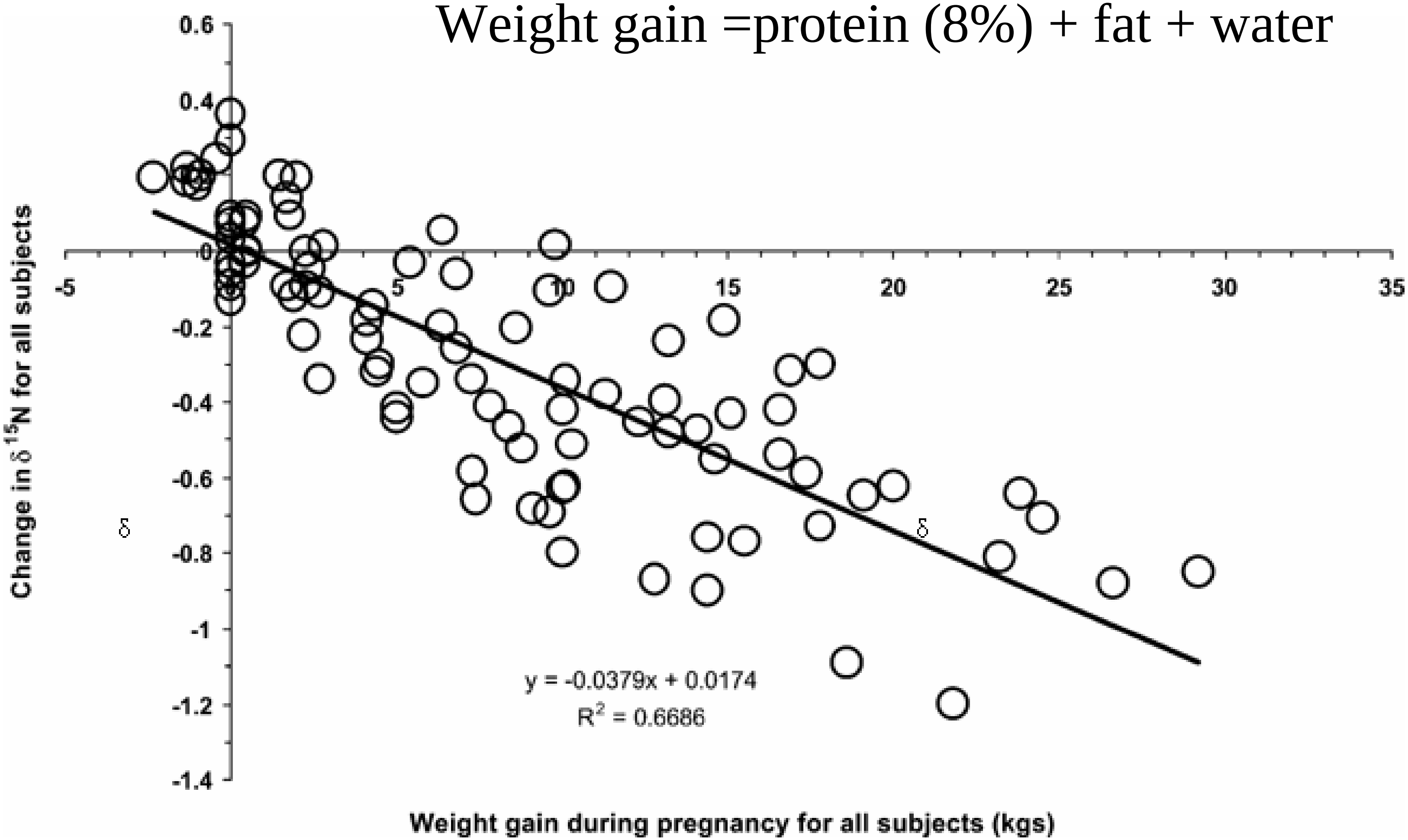
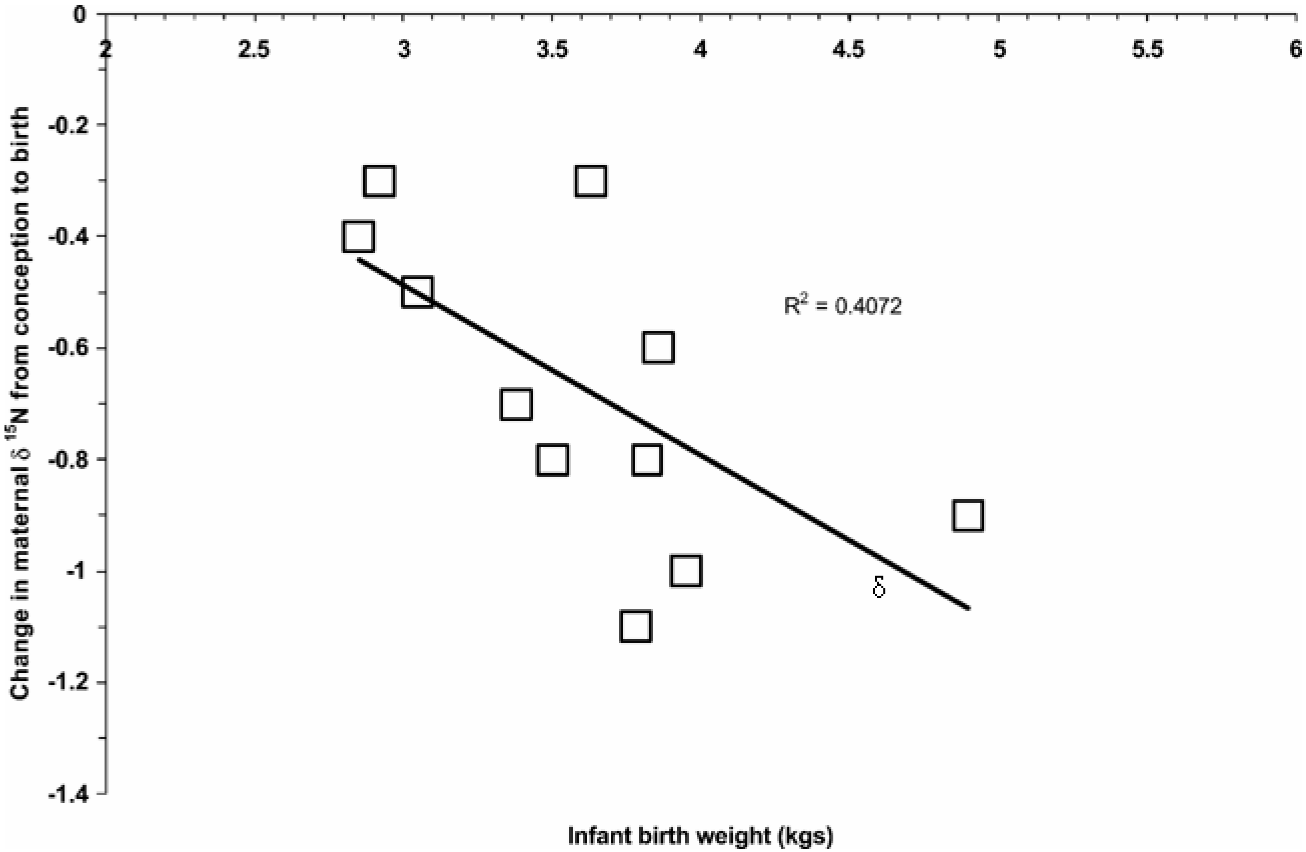


Figure 3. Changes in hair *d*[15]*N plotted against maternal weight gain during pregnancy for all 10 subjects. An inverse correlation is observed such that decreasing hair d*[15]*N values correspond to increases in weight and thus positive nitrogen balance.*



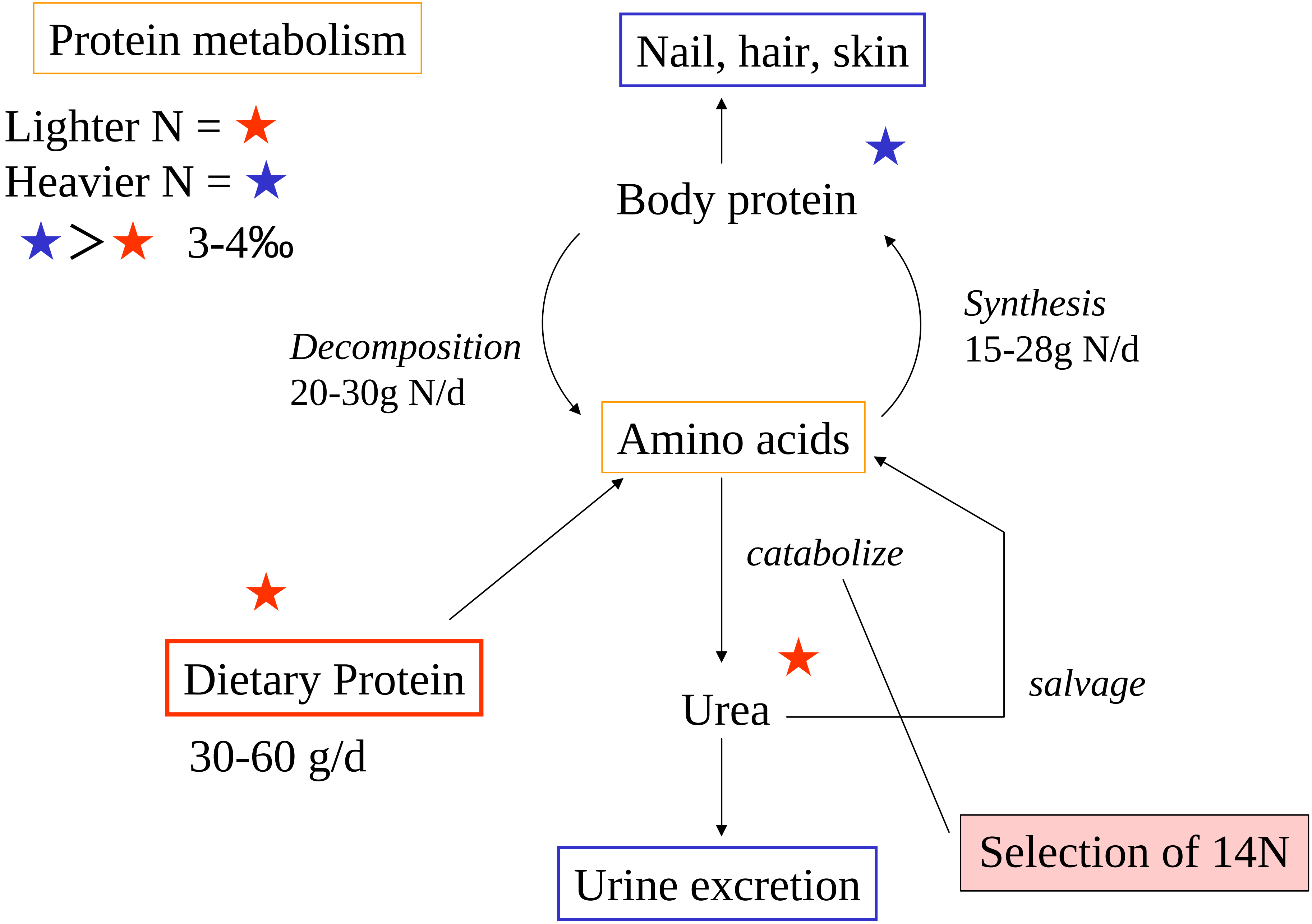
Fetus: 40% of total protein increase

Figure 4. Infant birth weight plotted against total change in maternal hair *d*^{15}*N* *from conception to birth.*

Summary of findings

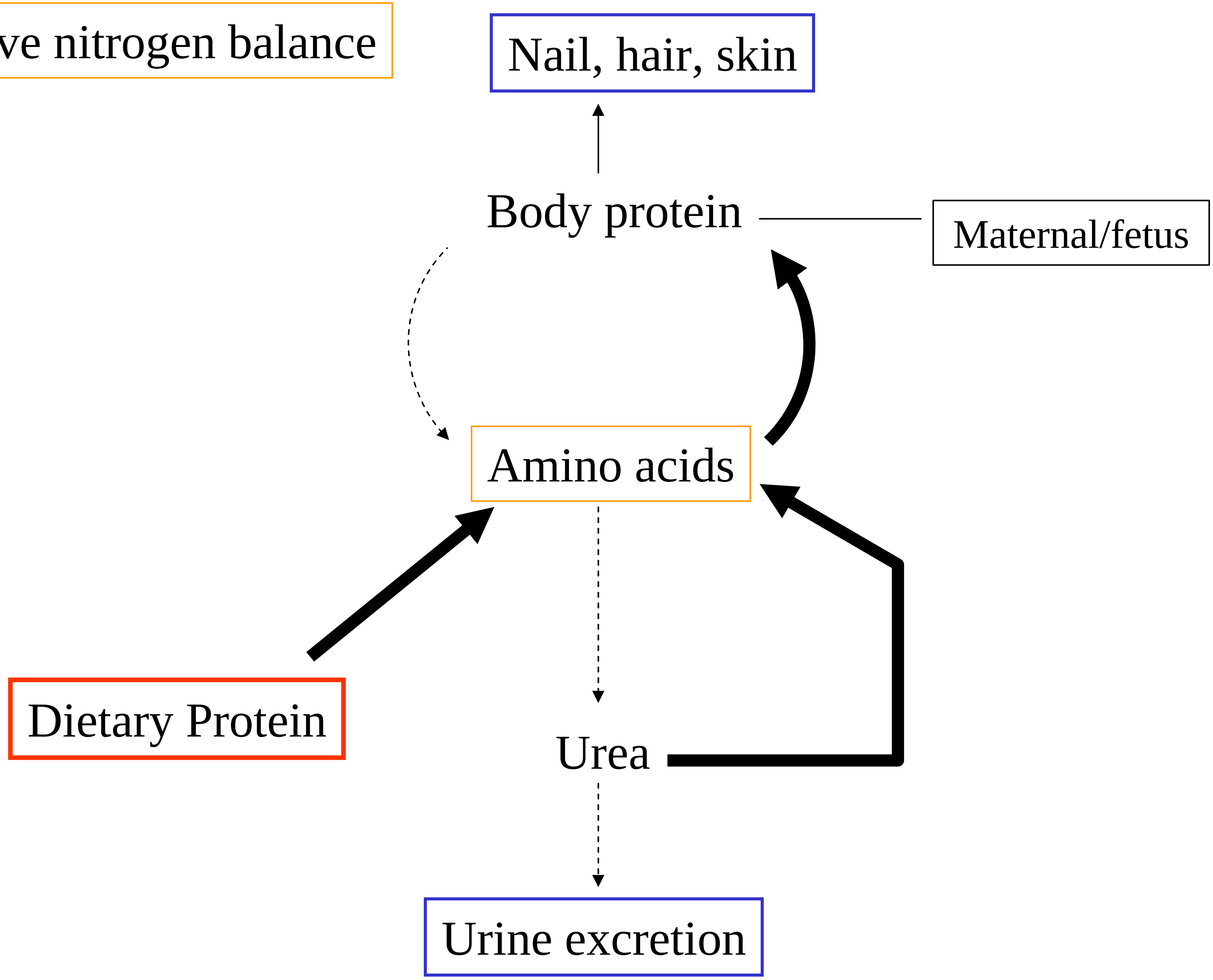
1. d15N decreased during the later stage of gestation.
2. Correlation was found between maternal weight gain and change in d15N (R^2=0. 67)
3. Correlation was found between infant birth weight and change in d15N (R^2=0.41).

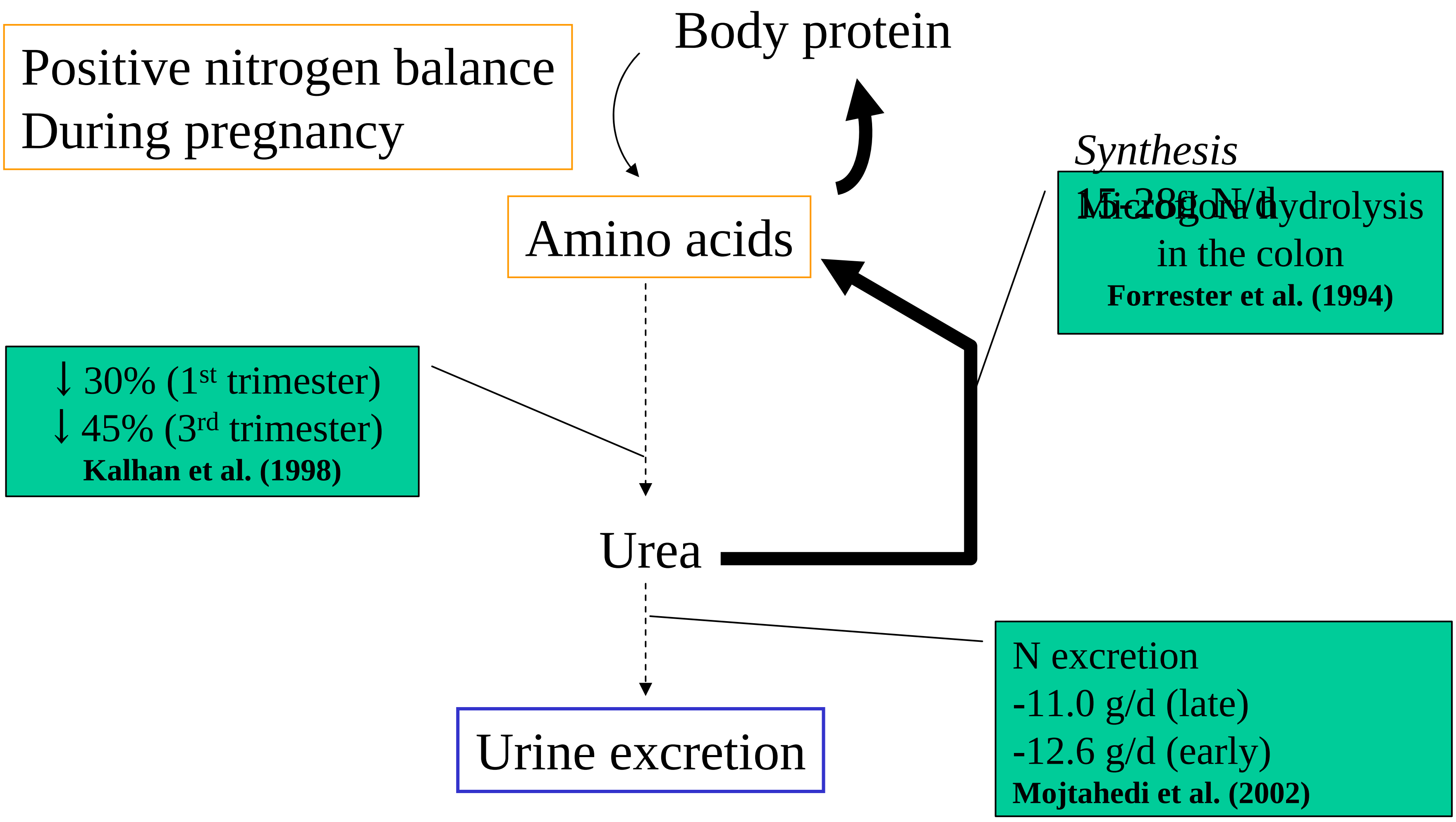
- Isotopic values of hair may be altered by the metabolic and physiological changes of pregnancy
- d15N decreased under the positive nitrogen balance.



Protein metabolism
Lighter N = ★
Heavier N = ★
★ > ★ 3-4‰
Nail, hair, skin
Body protein
Decomposition
20-30g N/d
Synthesis
15-28g N/d
Amino acids
Dietary Protein
30-60 g/d
catabolize
Urea
salvage
Urine excretion
Selection of 14N

Positive nitrogen balance

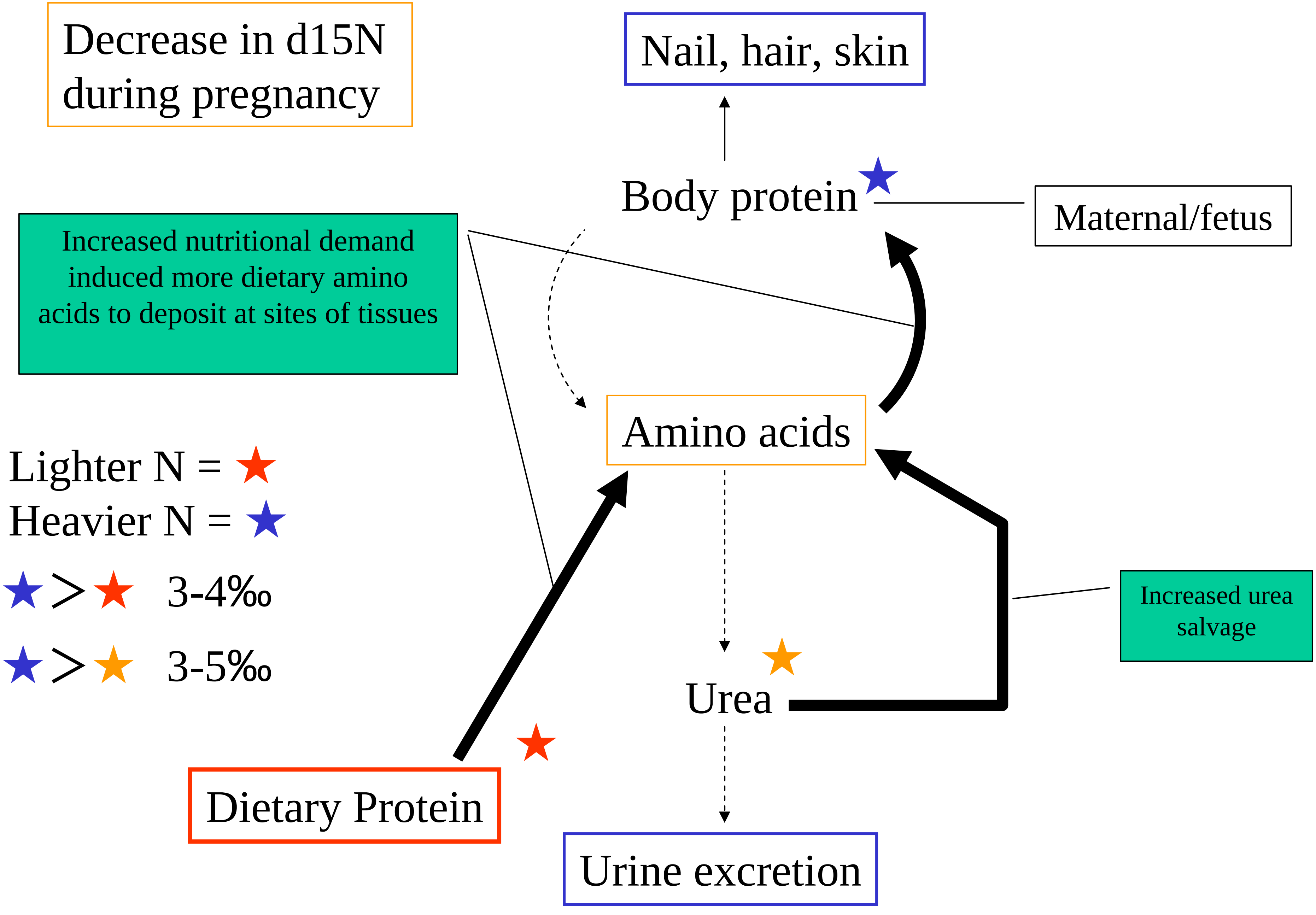




Biochemical mechanisms are not know.

The authors speculation:

1. Rise in circulation hormone (progesteron, estrogen) suppressed the enzymes of the urea cycle
2. Pregnancy-induced insulin resistance increased the glucose level in circulation, which means fewer amino acids need to be deaminated/transaminated


Decrease in d15N during pregnancy
Nail, hair, skin
Body protein
Maternal/fetus
Increased nutritional demand induced more dietary amino acids to deposit at sites of tissues
Amino acids
Lighter N =
Heavier N =
> 3-4‰
> 3-5‰
Increased urea salvage
Urea
Dietary Protein
Urine excretion

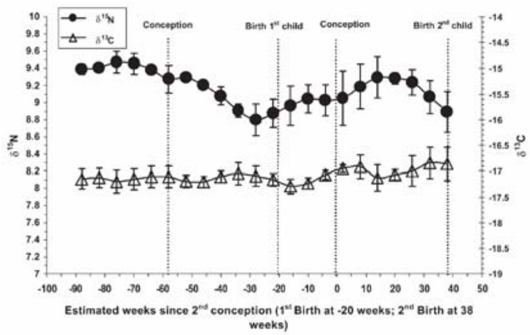
Possible mechanisms for the decrease in d15N during gestation

- Redirection of dietary amino acids from oxidation/excretion to tissue synthesis
- Increase in urea salvage

Lighter nitrogen will be utilized by the body

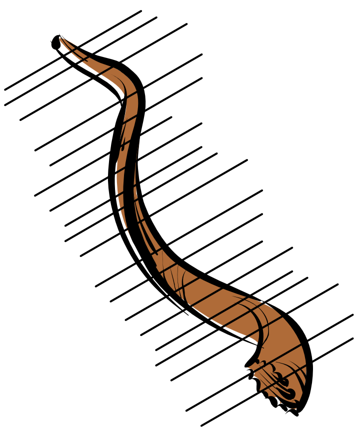
Potential application

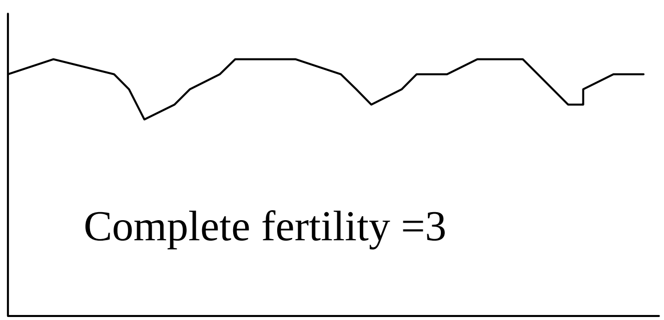
Palaeodietary and ecological studies:



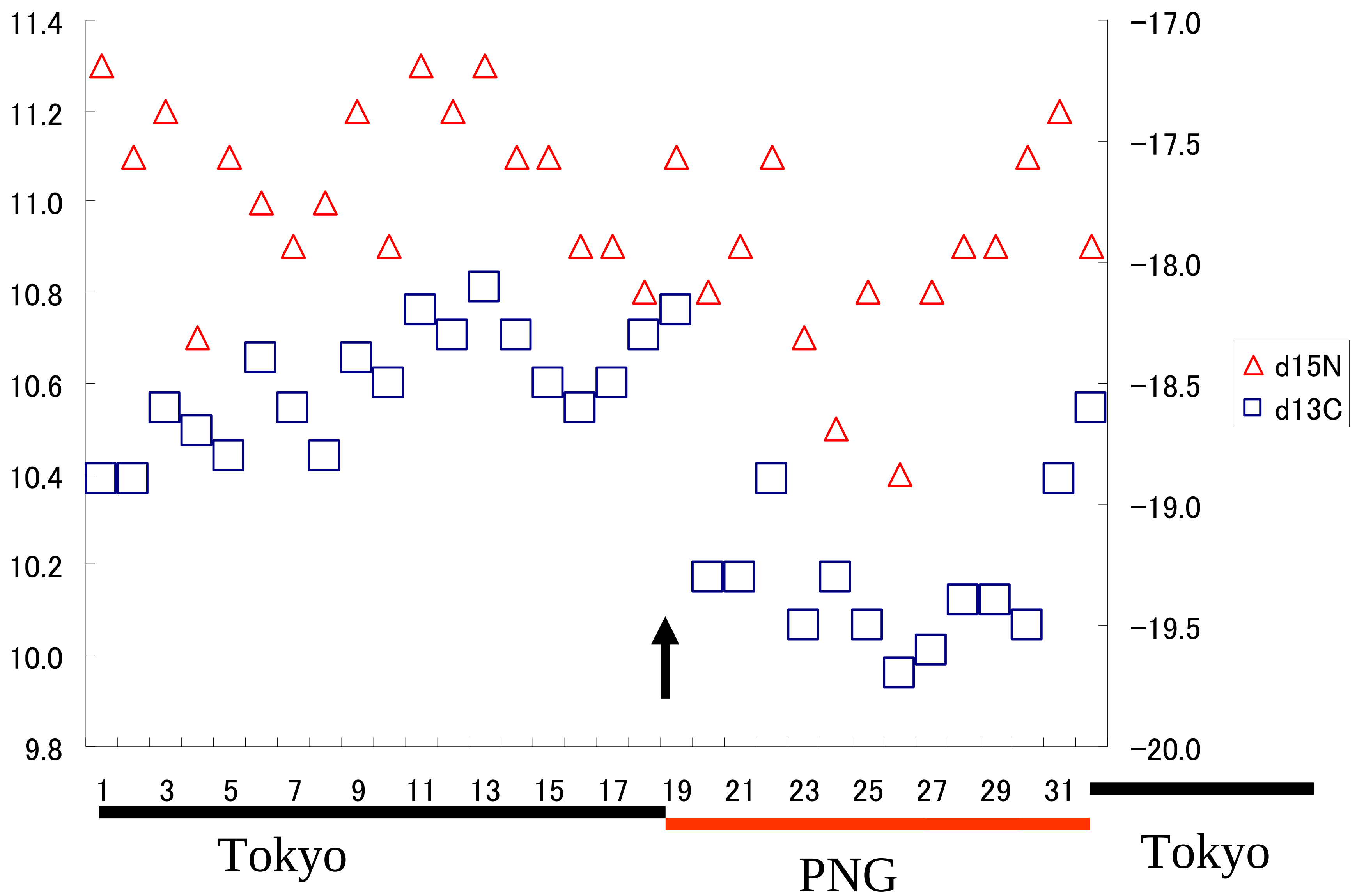
- Female d15N fluctuate with pregnancy; d15N difference by sex is due to diet and pregnancy.
- Female skelton d15N may be influenced by pregnancies.

Estimation of fertility using teeth, feathers, horns, etc.)

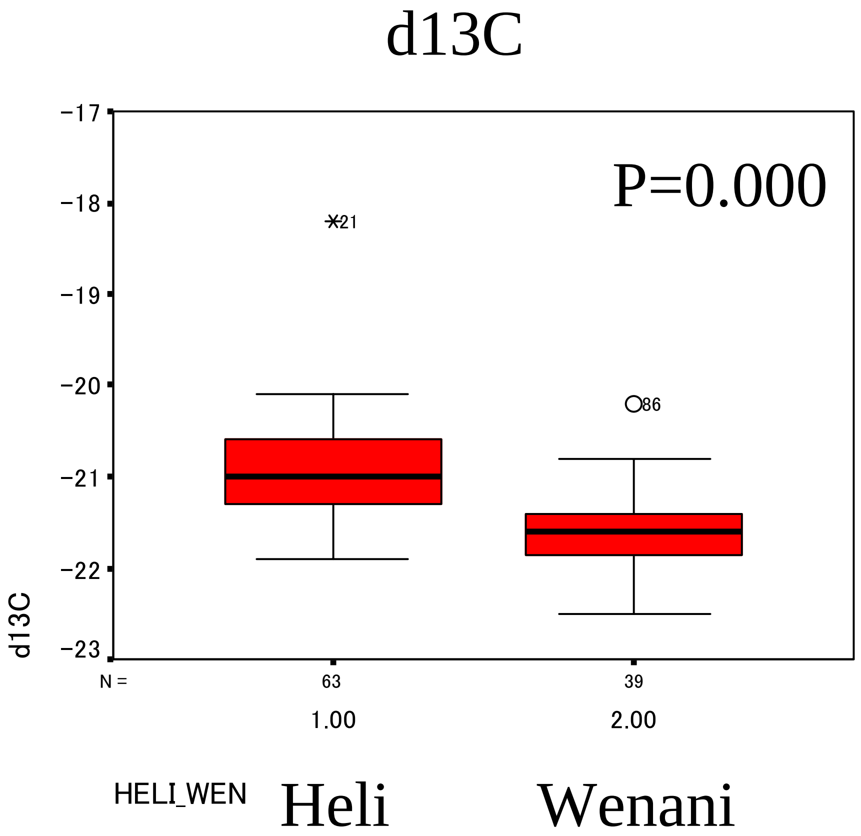




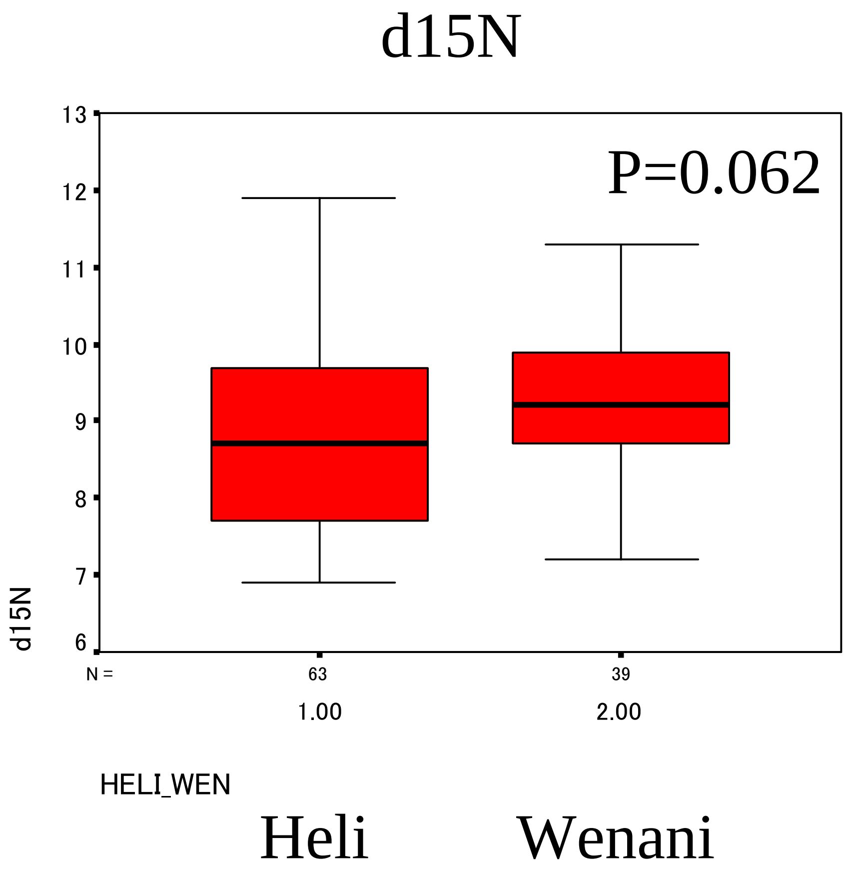
	Place of collection	
	Rural	Urban
PNG natives	101 Adaptation to low-protein intake	34 High-protein intake, sudden exposure
Japanese	10 Survival with low-protein intake	61 High-protein intake, usual



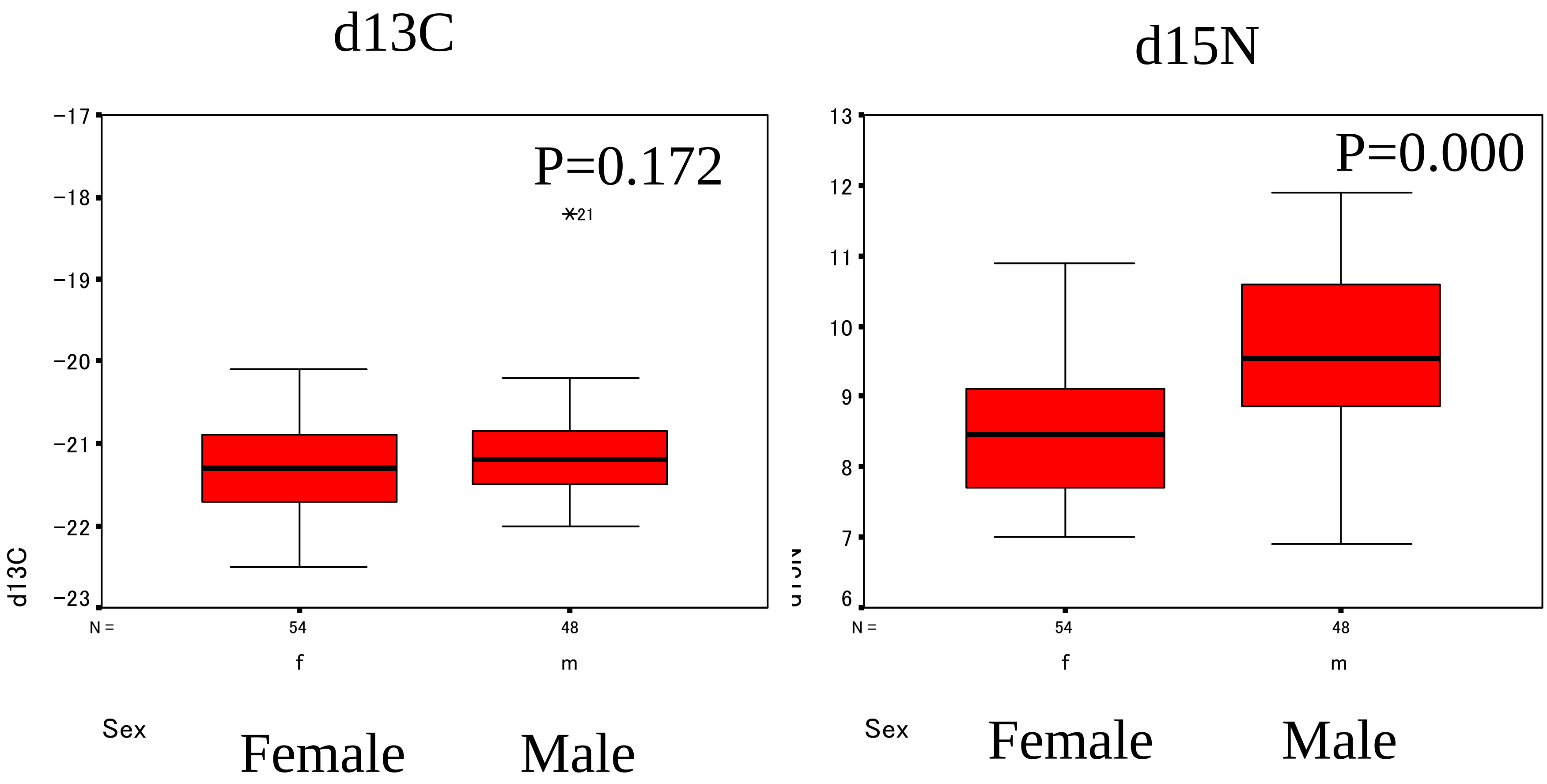
11.4
11.2
11.0
10.8
10.6
10.4
10.2
10.0
9.8
−17.0
−17.5
−18.0
−18.5
−19.0
−19.5
−20.0
d15N
d13C
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
Tokyo
PNG
Tokyo

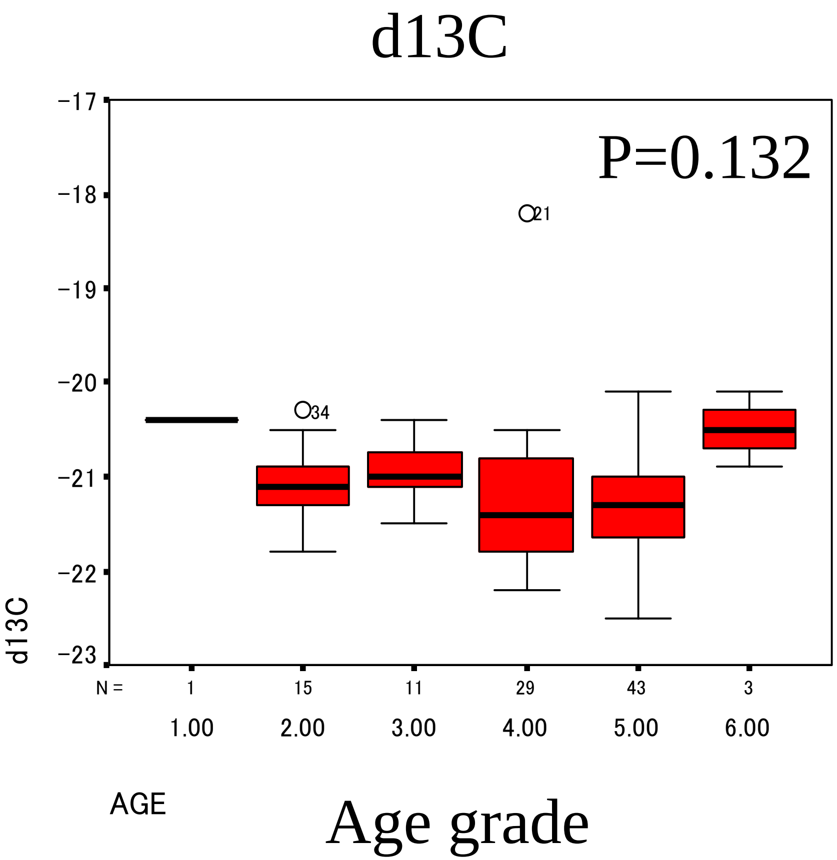


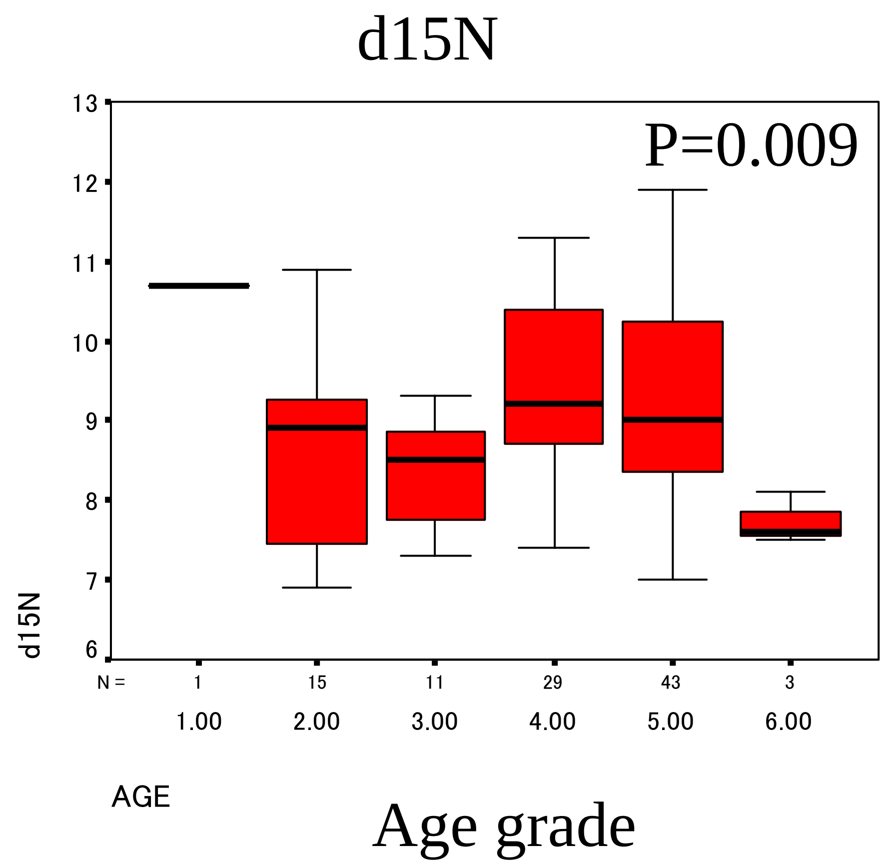
d13C
P=0.000
-17
-18
-19
-20
-21
-22
-23
d13C
21
86
N =
63
39
1.00
2.00
HELI_WEN
Heli
Wenani

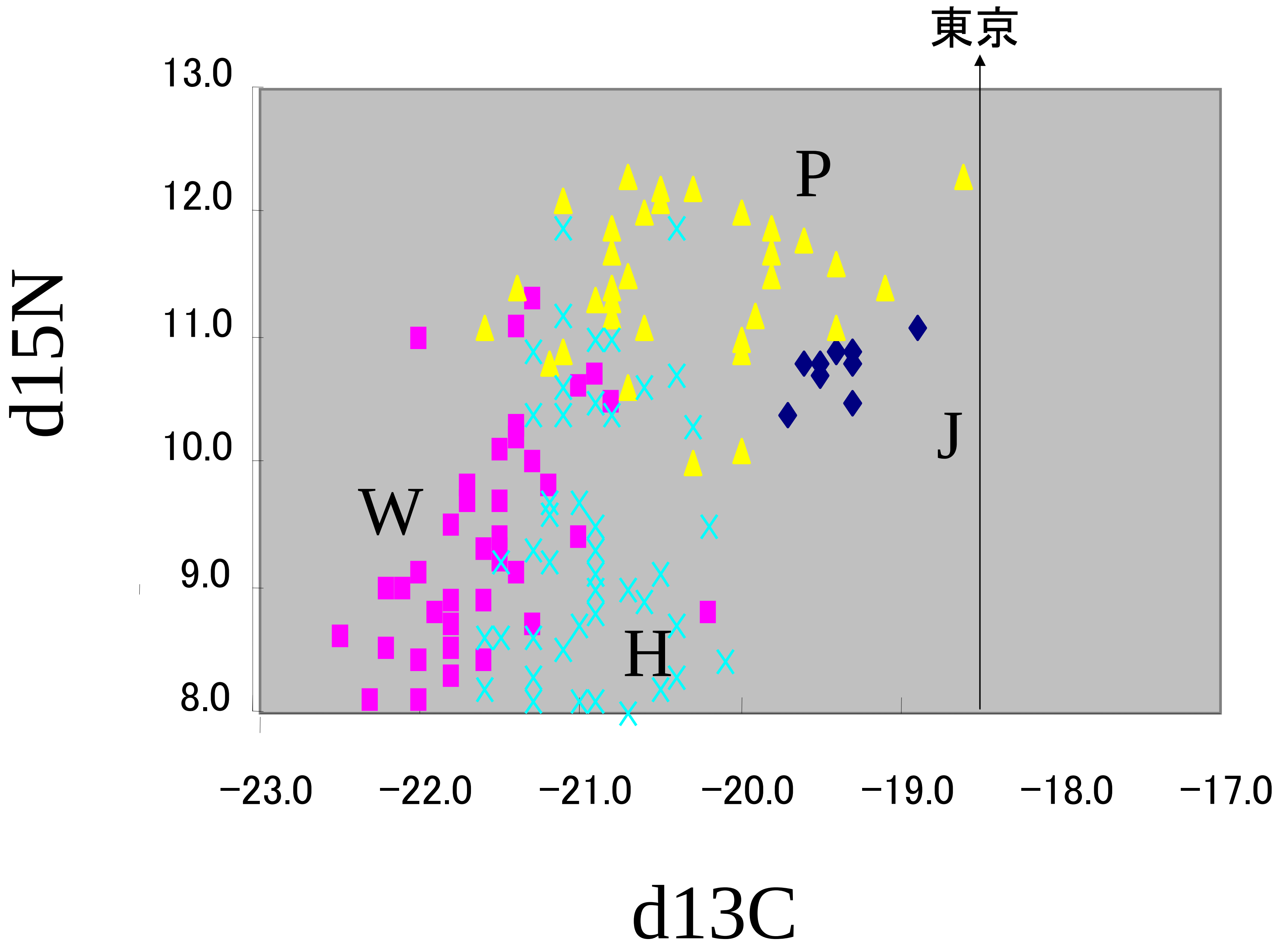


d15N
P=0.062
13
12
11
10
9
8
7
6
d15N
N =
63
39
1.00
2.00
HELI_WEN
Heli
Wenani


d13C
P=0.172
*21
-17
-18
-19
-20
-21
-22
-23
d13C
N =
54
48
f
m
Sex
Female
Male
d15N
P=0.000
13
12
11
10
9
8
7
6
d15N
N =
54
48
f
m
Sex
Female
Male


d13C
P=0.132
-17
-18
-19
-20
-21
-22
-23
d13C
○21
○34
N = 1 15 11 29 43 3
1.00 2.00 3.00 4.00 5.00 6.00
AGE
Age grade


d15N
P=0.009
13
12
11
10
9
8
7
6
d15N
N = 1 15 11 29 43 3
1.00 2.00 3.00 4.00 5.00 6.00
AGE
Age grade


東京
13.0
12.0
11.0
10.0
9.0
8.0
d15N
P
J
W
H
−23.0
−22.0
−21.0
−20.0
−19.0
−18.0
−17.0
d13C

d15N

Coefficients [a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	9.852	.522		18.874	.000
	SEX_N	−1.115	.212	−.465	−5.254	.000
	AGE	.127	.097	.122	1.310	.193
	HELI_WEN	.297	.229	.120	1.298	.197

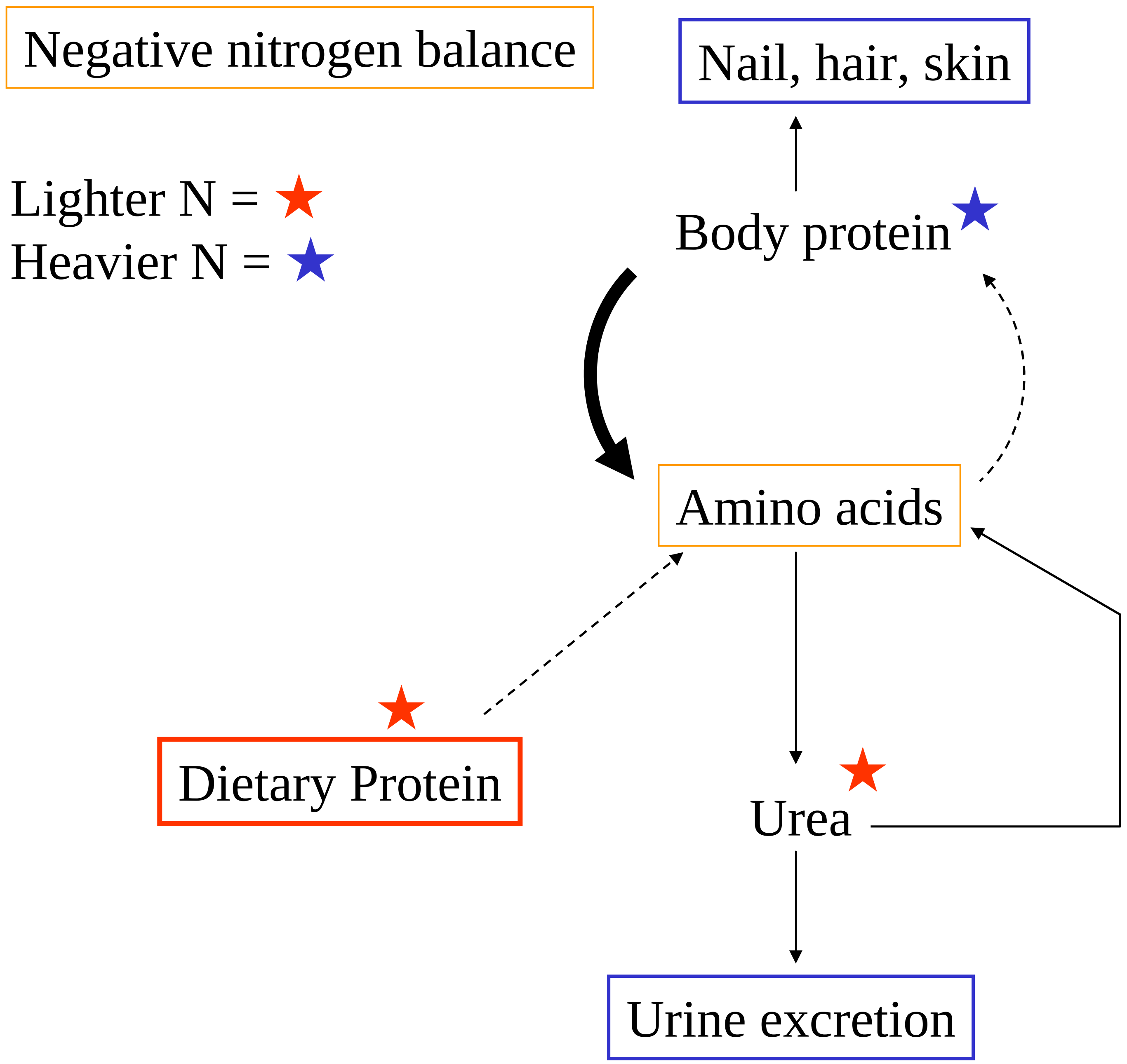
a. Dependent Variable: d15N

d13C

Coefficients [a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	−20.082	.251		−80.141	.000
	SEX_N	−.197	.102	−.162	−1.938	.056
	AGE	5.124E−02	.047	.097	1.099	.275
	HELI_WEN	−.723	.110	−.578	−6.589	.000

a. Dependent Variable: d13C



Negative nitrogen balance
Lighter N = ★
Heavier N = ★
Nail, hair, skin
Body protein
Amino acids
Dietary Protein
Urea
Urine excretion