

Expired $^{13}\text{CO}_2$ and Metabolic Fuel Mix in Young New Zealand Polynesian and Caucasian Women

Elaine C. Rush¹, Lindsay D. Plank² and W. Andrew Coward³

¹Department of Applied Science, Auckland Institute of Technology, Private Bag 92006, Wellesley Street, Auckland, New Zealand
Fax 64 9 3079973, E-mail elaine.rush@ait.ac.nz

²Department of Surgery, University of Auckland, Auckland, New Zealand

³Dunn Clinical Nutrition Centre, Hills Road, Cambridge, England

KEY WORDS Polynesian, Metabolism, Carbohydrate, Fat, Carbon 13.

ABSTRACT Polynesian New Zealanders have a high predisposition to obesity which may reflect an evolutionary development of more efficient fat deposition. We hypothesised that NZ Polynesian women would oxidise relatively more carbohydrate than their Caucasian counterparts both at rest and during exercise. Study subjects were 39 Polynesian and 40 Caucasian healthy female volunteers aged between 18 and 27 y with a wide range of fatness. Metabolic fuel mix was assessed from the respiratory exchange ratio (RER) and the proportion of ^{13}C in expired breath $^{13}\text{CO}_2/^{12}\text{CO}_2$ at rest and during three levels of exercise. Seven-day diet diaries were used to assess dietary intake of carbohydrate, fat and ^{13}C enriched sugars. Resting $^{13}\text{CO}_2/^{12}\text{CO}_2$ was significantly correlated with the proportion of enriched sugar in the dietary carbohydrate ($r = 0.34$, $p = 0.003$). Resting $^{13}\text{CO}_2/^{12}\text{CO}_2$ adjusted for enriched sugar intake was significantly correlated with RER ($R = 0.45$, $p = 0.001$). Ethnicity was not a significant predictor of adjusted $^{13}\text{CO}_2/^{12}\text{CO}_2$ at rest or during exercise, whereas degree of central adiposity, measured as the subscapular-to-triceps skinfold ratio, and percentage body fat were significant. Differences in metabolic fuel mix were accounted for by differences in the diet, body fat and distribution of the body fat.

INTRODUCTION

In simple terms weight gain will only occur if there is a long term excess of energy intake over energy expenditure. Any differences between the nutrients being oxidised as fuel and the proportions of these nutrients in the diet imply changes in body composition even under conditions of nutrient balance. De novo lipogenesis (Flatt et al., 1985; Acheson et al., 1987) does not appear to be an important factor in relation to weight gain. It follows that fat intake must equal fat oxidation for body fat stores to remain constant.

It has been hypothesised (Wade et al., 1990) that high rates of carbohydrate utilisation may

predispose a person to excess fat storage. Further it has been suggested (McGarvey, 1991; Dowse et al., 1992) that in Polynesian people, a high predisposition to obesity may reflect an evolutionary development in energy storage metabolism resulting in the conservation of energy during famines and efficient fat deposition in times of food abundance. This "thrifty genotype" originally hypothesised by Neel (1962) may in parallel with environmental changes in diet and physical activity help explain the increasing and greater prevalence of obesity in New Zealanders of Polynesian origins. In other words, genetically determined differences in metabolic fuel mix in association with a high fat, low exercise environment will favour the development of obesity (Ravussin, 1995).

We hypothesised that if Polynesian women exhibit this aspect of the thrifty genotype they would oxidise relatively more carbohydrate than Caucasian both at rest and during exercise. We assessed metabolic fuel mix in two ways. Firstly, by measuring the respiratory exchange ratio (the ratio of expired carbon dioxide to oxygen uptake) under fasting conditions and secondly, from the proportion of ^{13}C in expired breath (Barstow et al., 1989) with account taken of enriched carbohydrate intake in the diet.

METHODS

Subjects

The study was approved by the University of Auckland Human Subjects Ethics committee and the Auckland Institute of Technology Ethics Committee. Study subjects were 82 healthy female volunteers aged between 18 and 27 y,

selected for ethnicity and body size by personal contact and advertisement. All subjects gave their free and informed consent. Measured resting blood pressure and fasting blood glucose were within normal limits for all subjects, i.e., diastolic blood pressure and fasting blood glucose were within normal limits for all subjects, i.e., diastolic blood pressure was not > 90 mm Hg and fasting blood glucose was < 5.7 mmol/L. Forth-two identified themselves as New Zealand European, 40 as Polynesian (22 Samoan, 12 Maori, 3 Tongan, 2 Niuean and 1 Cook Islander). Twenty-one and 20 in these respective ethnic groups had a BMI > 30.

Protocol

Two weeks prior to the present investigation measurements were made of resting blood pressure and fasting blood glucose and ^{18}O labelled water was administered orally. At this time standing height was measured to 0.1 cm with a stadiometer, weight was measured to 0.025 kg on a beam balance with the subject in minimal clothing. Skinfold thicknesses were obtained. Weight was checked for stability seven days later. Subjects arrived by the car at the exercise laboratory in the morning after an overnight fast. They were asked to refrain from exercise on that morning. Water was allowed ad libitum. The laboratory was air-conditioned and at a constant temperature of 21.5°C. Barometric pressure and humidity were measured before each set of measurements. Subjects were familiarised with the required procedure and briefly walked on the motor-driven, variable speed treadmill (Quinton Instrument Co., Seattle, Washington) to choose a comfortable walking speed which was usually between 3 and 4.5 km/hour. Heart rate was monitored using a portable cardiometer. Sport Tester PF3000 (Polar electro, Kempele, Finland). The walking exercise protocol was devised so that all subjects experienced approximately the same exercise stress and that their heart rate was kept below 135 beats per minute (bpm) to avoid a lactic acidosis. In healthy, sedentary individuals blood lactate rises above resting values when the exercise intensity exceeds 50-60% of maximum oxygen consumption or a heart rate of 60-70% of maximum (Wilmore and Costill, 1994). The predicted maximum heart rate for women

aged 27 was determined using the Lange-Anderson (Lange-Anderson et al., 1971) formula:

Maximum heart rate = $210 - (0.65 \times \text{age in years})$ beats per minute.

A conservative maximum allowable heart rate of 135 bpm was chosen for all subjects so that potential discomfort was minimised especially for the larger, unfit subjects. Cardiorespiratory measurements were made throughout the test. The subjects wore a noseclip and breathed through a mouthpiece connected via a two way Koegel respiratory valve (Ewald Koegel Co., TX) to an open respiratory system. Room air was inspired through the valve and expired gas was directed through a manifold with taps to five Douglas bags. The subject initially sat on a chair on the treadmill and listened to quiet music with the noseclip and mouthpiece in place. The measurement protocol started when the heart rate had been stable for at least ten minutes. Four further minutes of stable heart rate were recorded before the first five-minute expired air collection into a Douglas bag was obtained, followed one minute later by another five-minute collect into the next Douglas bag. The subject then was asked to stand and walk on the treadmill at the individually predetermined speed for six minutes on the level (exercise 1) and then six minutes each at two gradients (exercise 2 and 3). The two gradients were chosen empirically for each subject so that the heart rate increased with the final heart rate less than 135 bpm. A stable state at each exercise level is achieved after 4 minutes (Shephard, 1982) and expired gas was collected for the last two minutes at each level. All measurements on all subjects were made by the same investigator.

Anthropometry and Body Composition

Skinfold thicknesses (triceps, biceps, subscapular and suprailiac) were measured as the average of triplicate measures using Harpenden calipers and standard techniques (Durnin and Womersley, 1974). Total body water (TBW) was determined by stable isotope (oxygen-18) dilution using the multipoint slope/intercept method as described by Coward (Coward et al, 1994). An initial dose of 1.5g/kg of 10.16% H_2^{18}O (Enritech, Weizmann Institute of Science, Rehovot, Israel) of fat free mass predicted from the anthropometric measurements was given. Timed urine samples

were collected four and five hours after dose administration and then 2, 5, 7 and 14 days later. These were the points used to determine the oxygen-18 dilution space which was divided by 1.01 to give TBW. Fat mass (FM in kg), fat free mass (FFM in kg) and percentage fat were determined using the assumption that FFM is 73 per cent water.

Respiratory Exchange Ratio (RER)

The collected expired gas was sampled sequentially from each bag via a small tube containing granulated anhydrous magnesium perchlorate. The dried gas was then passed at 200 mL/min through, in turn, a paramagnetic oxygen analyser (Servomex OA570, Taylor Instrument Analytics Ltd, Crowborough, Sussex, England) and then an infrared carbon dioxide analyser (Medical Gas Analyser LB2, Beckman Instruments Ltd IL, USA). The gas analysers were calibrated against known gas standards (5.00% CO_2 and 14.90% O_2), pure nitrogen, and fresh air (0.03% CO_2 and 20.94% O_2). The volume of gas in each Douglas bag was measured by drawing the gas through a coal gas meter. This meter was calibrated and checked for linearity against a Tissot bell spirometer. Volumes sampled for gas analyses were added to the volumes measured. Oxygen uptake (VO_2) and carbon dioxide output (VCO_2) at standard temperature and pressure dry (STPD, 0°C, 760 mmHg) were calculated from the parameters measured and the RER derived. VO_2 and VCO_2 at rest were taken as the average of the two five-minute collections. Based on these duplicate measurements the precision (coefficient of variation) for RER was 4.6%.

Exercise Intensity

The intensity of exercise for each subject was determined by dividing the exercise VO_2 by the averaged resting VO_2 .

$^{13}\text{CO}_2/^{12}\text{CO}_2$ Ratio

Subsamples of 350 mL of expired gas were taken from each Douglas bag for later analysis of the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio. These samples were stored for as short a time as possible in foil bags with a tap. Testing the gas composition in one bag over a number of days showed no change. The ratio was determined using isotope ratio mass spectrometry (Tracermass, Europa Scientific

Inc., Crewe, England). It is standard practice to measure ^{13}C isotopic abundances against a defined standard. All values of the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio are expressed as either positive or negative per mil (‰) relative differences (delta units) from the standard according to the formula:

$^{13}\text{CO}_2/^{12}\text{CO}_2$ (delta units) = $1000 \times (\text{Ru} - \text{Rs}) / \text{Rs}$ where Ru, Rs refer to the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio for the unknown sample and the defined standard, respectively. The reference standard is Pee Dee Belemnite Limestone. The coefficient of variation for the measurement of the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio as estimated from analysis of the duplicate Douglas bags taken at rest was 1%.

Nutrient Intake

Self-reported seven-day food diaries, using household measures, were kept by the subjects during the first seven days of energy expenditure measurement by doubly-labelled water. The subjects were carefully instructed on how to fill in the diary and the diary was reviewed with them at the end of the seven-day collection period. Following guidelines reviewed by Mackerras (Mackerras, 1991). The energy-producing food components of relevance to this study were protein, fat, available carbohydrate and ethylalcohol. To quantitate these components a Macintosh computer programme (Diet Balancer™) was used with food composition data manually entered from the NZ Food Tables (Burlingame et al, 1994). New Zealand energy conversion factors were used to convert grams of nutrient to energy. The good quotient (FQ) was calculated for each subject from the proportion of total energy intake derived from each of the macronutrients: Protein, carbohydrate, fat, and alcohol (Black et al., 1986).

Ratio of Enriched to Total Carbohydrate

As maize and cane sugars are enriched in ^{13}C (Schmidt et al., 1986; Schoeller et al., 1980) compared to other dietary components (-12 vs -22‰) an analysis of any differences in the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio needs to take individual dietary sources of carbon into account. In New Zealand all readily available sucrose is derived from the four-carbon sugars maize and cane. In foods containing carbohydrate from maize and cane the proportion of enriched carbohydrate in the total weight of each food was determined using estimates based on

the food tables, e.g., cakes and biscuits were estimated as 25g/100g of the food. Using these proportions the seven-day food diary for each subject was analysed to derive a ratio of amount of enriched carbohydrate to total carbohydrate intake ($C_6CHO/\text{total CHO}$).

Statistical Analysis

All data in the text are expressed as means $\pm$ standard deviation (SD) unless otherwise stated. The Pearson correlation coefficient and multiple linear regression analysis were used to investigate relationships between selected variables. Student's *t* test and analysis of variance (ANOVA) were used where appropriate to compare ethnic groups. Statistical significance was set at $p < 0.05$. Analysis were carried out using the Statview 512+™ software package (Abacus Concepts Inc., CA, USA) and PC-SAS v.6.04 (SAS Institute, Cary, NC, USA).

RESULTS

Of the 82 subjects recruited 3 subjects were excluded from the analysis because of failure to return food diaries (one Polynesian, one Caucasian) and an excessively high result for the resting breath analysis of the $^{13}CO_2/^{12}CO_2$ ratio (one Caucasian). Physical characteristics (Table 1) were similar for the two ethnic groups except that the Polynesian women had significantly higher fat-free mass ($p = < 0.0001$), and fasting blood glucose concentration ($p = 0.0016$).

As expected the intensity of exercise as a multiple of resting oxygen uptake increased at each level as did RER (Table 2). The Polynesian women exercised at a greater multiple of their resting oxygen uptake during exercise 1 than the Caucasian women but there was no significant difference in RER between the groups at rest or during exercise. No statistically significant cor-

Table 1: Physical characteristics of the Polynesian and Caucasian women*

Characteristic	Polynesian (n = 39)		Caucasian (n = 40)	
Height (cm)	166.5 $\pm$ 6.2	(153.4 - 178.2)	164.8 $\pm$ 6.4	(153.0 - 180.2)
Weight (kg)	84.7 $\pm$ 18.3	(56.2 - 131.7)	78.2 $\pm$ 22.6	(48.1 - 38/3)
Age (y)	22 $\pm$ 2	(18 - 25)	22 $\pm$ 2	(18 - 27)
BMI (kg/m ²)	30.7 $\pm$ 7.5	(19.8 - 51.8)	28.7 $\pm$ 7.6	(16.5 - 48.0)
Body fat (%)	38.8 $\pm$ 7.4	(26.0 - 54.0)	40.2 $\pm$ 10.0	(22.2 - 59.3)
Fat mass (kg)	34.0 $\pm$ 13.5	(15.6 - 71.1)	33.4 $\pm$ 17.3	(10.9 - 82.0)
Fat-free mass (kg)	50.7 $\pm$ 6.1	(37.0 - 64.1)	44.9 $\pm$ 6.8 ^b	(30.5 - 57.5)
Subscapular/triceps ratio	1.12 $\pm$ 0.27	(0.64 - 1.77)	0.85 $\pm$ 0.25 ^b	(0.38 - 1.45)
Fasting glucose (mmol/L)	4.3 $\pm$ 0.5	(2.9 - 5.3)	3.9 $\pm$ 0.6 ^b	(2.9 - 5.1)
Systolic blood pressure (mmHg)	117 $\pm$ 6	(105 - 130)	120 $\pm$ 9	(105 - 140)
Diastolic blood pressure (mmHg)	77 $\pm$ 6	(65 - 88)	78 $\pm$ 6	(65 - 90)
Resting heart rate (bpm)	71.5 $\pm$ 11.0	(51 - 96)	74.9 $\pm$ 11.0	(51 - 100)

* Mean $\pm$ s.d.; range in parentheses. BMI, body mass index.

^b Significantly different from Polynesians. $p < 0.002$ (two-sample independent *t* test).

Table 2: Respiratory exchange ratio (RER) and exercise intensity as a multiple of resting oxygen uptake at rest and during exercise for Polynesian and Caucasian women*

	RER			Exercise intensity		
	Polynesian (n = 39)	Caucasian (n = 40)	<i>p</i> ^b	Polynesian (n = 39)	Caucasian (n = 40)	<i>p</i> ^b
Rest	0.79 $\pm$ 0.06	0.81 $\pm$ 0.07	0.30			
Exercise 1	0.81 $\pm$ 0.05	0.81 $\pm$ 0.06	0.98	3.86 $\pm$ 0.61	3.52 $\pm$ 0.53	0.01
Exercise 2	0.84 $\pm$ 0.06	0.85 $\pm$ 0.05	0.83	4.77 $\pm$ 0.75	4.48 $\pm$ 0.60	0.07
Exercise 3	0.87 $\pm$ 0.05	0.88 $\pm$ 0.08	0.75	5.62 $\pm$ 1.09	5.41 $\pm$ 1.04	0.38

* mean $\pm$ s.d.

^b *t* test for comparison of ethnic groups

relations were seen between the intensity of exercise and percentage body fat indicating that all subjects were exercised to similar levels.

Figure 1 shows the changes with exercise level of the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio in expired breath for the two ethnic groups. A two-factor (ethnicity and level of exercise) repeated-measures ANOVA demonstrated that the pattern of change of $^{13}\text{CO}_2/^{12}\text{CO}_2$ with exercise was the same in both groups ($p = 0.51$). For both groups the ratio fell significantly during the fifth and sixth minutes of exercise 1, when the sample was taken, and rose again as the work rate and time increased ($p < 0.0001$). At all exercise levels the ratio was lower for the Caucasian women, although not reaching statistical significance ($p = 0.08$).

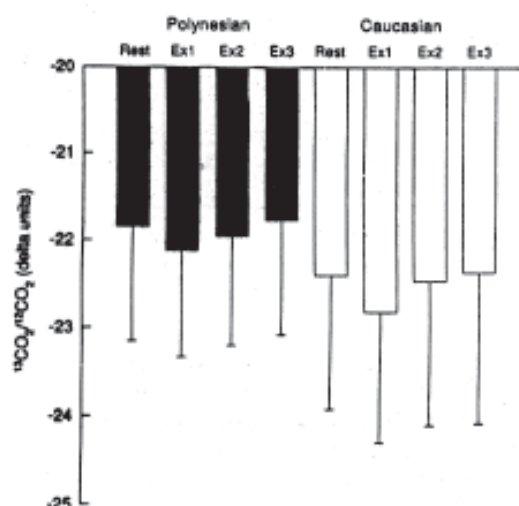


Fig. 1. $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio for 39 Polynesian and 40 Caucasian women measured in expired breath at rest and during three levels of exercise (mean $\pm$ s.d.)

The two measures of metabolic fuel mix, RER and $^{13}\text{CO}_2/^{12}\text{CO}_2$, were uncorrected of the Polynesian groups at all levels of exercise (Table 3) but significantly correlated for the Caucasian group except at exercise level 1.

For the Caucasian women, percentage body fat was positively related to the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio at exercise levels 2 and 3 (Correlation co-efficients

0.31 and 0.33, respectively). For the Polynesian women at the three levels of exercise percentage body fat and $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio were not significantly correlated.

Table 3: Correlation coefficients (r) between enrichment of breath CO_2 and respiratory exchange ratio at rest and during exercise for 39 Polynesian and 40 Caucasian women

	Polynesian		Caucasian	
	r	p	r	p
Rest	0.14	0.41	0.43	0.005
Exercise 1	-0.14	0.39	0.17	0.29
Exercise 2	-0.03	0.87	0.35	0.03
Exercise 3	-0.04	0.80	0.32	0.047

In table 4 the diet diary analysis of energy intake is summarised and the percentage of energy provided by protein, carbohydrate, fat and alcohol shown. Three were very significant differences between the ethnic groups with reported total energy intake ($p = 0.0003$) and the percentage of total energy intake supplied by fat ($p = 0.007$) in the Caucasian group being less than that for the Polynesians. Energy intake from fat was 1.0 MJ/day higher in the Polynesian group ($p = 0.0001$). For the Polynesian women there was a positive relationship between the percentage of energy intake derived from fat and total daily energy intake ($r = 0.58$, $p < 0.0001$). This relationship was not as strong for the Caucasian group ($r = 0.36$, $p = 0.02$). Twenty-four (9 Polynesian, 15 Caucasian) of the 79 subjects drank alcohol (20 ± 56 , range 7–413 g per 7 days). Table 4 also shows that the proportion of carbohydrate intake that was in the form of simple, C_4 enriched sugar and the FQ were similar for the two ethnic groups.

The proportion of enriched sugar in the dietary carbohydrate was significantly related to resting breath $^{13}\text{CO}_2/^{12}\text{CO}_2$ ($r = 0.33$, $p = 0.003$). Adjustment of the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio for the intake of enriched sugar by multiple regression analysis produced a stronger relationship between $^{13}\text{CO}_2/^{12}\text{CO}_2$ and the RER ($r = 0.45$, $p = 0.0002$) than when $^{13}\text{CO}_2/^{12}\text{CO}_2$ was regressed on RER alone ($r = 0.28$, $p = 0.01$). Ethnicity was not a significant predictor ($p = 0.13$) when included in the multiple regression analysis.

Table 5 details the results of multiple regression analyses for predictors of breath $^{13}\text{CO}_2/^{12}\text{CO}_2$.

Table 4: Diet-diary analysis of macronutrient intake for Polynesian and Caucasian women^a

	Polynesian (n = 39)		Caucasian (n = 40)	
Total energy intake (Mj-day)	9.53 ± 2.46	(3.86 - 14.52)	7.75 ± 1.64 ^b	(3.88 - 10.80)
Protein (% of total energy intake)	14.3 ± 4.1	(9.9 - 34.6)	14.9 ± 2.7	(11.4 - 23.8)
Carbohydrate (% of total energy intake)	46.3 ± 6.0	(34.2 - 62.0)	48.7 ± 5.5	(33.2 - 60.2)
Fat (% of total energy intake)	38.5 ± 5.9	(20.9 - 47.2)	35.1 ± 5.1 ^b	(17.7 - 42.9)
Alcohol (% of total energy intake)	0.9 ± 2.7	(0 - 15.4)	1.3 ± 2.4	(0 - 9.9)
C ₄ CHO/total CHO ^c	0.19 ± 0.08	(0.02 - 0.38)	0.15 ± 0.08	(0.02 - 0.38)
Food Quotient	0.858 ± 0.017	(0.829 - 0.907)	0.866 ± 0.016 ^b	(0.830 - 0.904)

^amean ± s.d.; range in parentheses.^bSignificantly different from Polynesians, *p* < 0.05 (two-sample independent *t* test).^cRatio of four-carbon enriched carbohydrate to total carbohydrate.**Table 5:** Results of regression analysis with ¹³CO₂/¹²CO₂ ratio as dependent variable and enriched sugar intake, ethnicity, percentage body fat, and logarithm of subscapular to triceps skinfold ratio as independent variables for 39 Polynesian and 40 Caucasian women at rest and during exercise.

C ₄ CHO/totalCHO ^a	Regression coefficients			R ²	SEE	p	
	Ethnicity ^b	Body fat(%)	log STR				
Intercept							
<i>Rest</i>							
5.276 ± 1.882 ^{c,d}	0.361 ± 0.316			-23.203 ± 0.361	0.13	1.37	0.006
6.060 ± 1.786 ^c		0.043 ± 0.017 ^d		-24.850 ± 0.776	0.18	1.33	0.0005
4.831 ± 1.855 ^d			2.455 ± 1.179 ^e	-22.885 ± 0.360	0.16	1.34	0.001
<i>Exercise 3</i>							
7.107 ± 1.969 ^c	0.347 ± 0.332			-23.460 ± 0.381	0.18	1.43	0.0006
7.955 ± 1.790 ^c		0.063 ± 0.017 ^e		-25.923 ± 0.783	0.29	1.33	<0.0001
6.296 ± 1.903 ^c			3.316 ± 1.210 ^d	-23.058 ± 0.371	0.24	1.38	<0.0001

SEE = model standard error of estimate; STR = subscapular to triceps skinfold ratio ^aratio of four-carbon to total carbohydrate;^b dummy codes are 1 and 0 for Polynesian and Caucasian subjects, respectively; ^cestimated regression coefficient ± standard error of estimate; ^d *p* < 0.02; ^e *p* < 0.005; ^f *p* < 0.05.

ratio both at rest and during exercise level 3) after adjustment for the effect of enriched sugar intake. Ethnicity was not a significant predictor while percentage body fat and the logarithm of the subscapular to triceps skinfold ratio were significant both at rest and during exercise. At rest, liver glycogen is the dominant source of carbohydrate and after exercising for 15 minutes it is more likely that the carbohydrate being burnt would be muscle glycogen. Muscle glycogen has a slower turnover rate than liver glycogen and therefore would better reflect long-term averaged intake of enriched carbohydrate. The relationship between ¹³CO₂/¹²CO₂ for exercise 3, reported intake of sugar and ethnicity accounted for five percent more of the variance (*R*² = 0.18) in the delta value than the model at rest (*R*² = 0.13). When the logarithm of the subscapular to triceps skinfolds ratio replaced ethnicity in the model a better fit (*R*² = 0.24) was obtained. The variance explained rose to 29% when percentage body fat replaced the

subscapular to triceps skinfold ratio as a predictor and the positive regression co-efficient indicated that the fatter women burnt more carbohydrate. Thus it was not ethnicity that determined fuel mix but percentage body fat and body fat distribution.

DISCUSSION

The present study was designed to explore the hypothesis that New Zealand women of Polynesian origin oxidise relatively more carbohydrate at rest and during exercise than their counterparts of European origin. The metabolic fuel mix was assessed by measuring the respiratory exchange ratio and the ¹³C enrichment of expired breath carbon dioxide. Analysis of dietary intake allowed account to be taken of the relative proportion of enriched sugar in the dietary carbohydrate.

The natural enrichment of breath CO₂ with ¹³C at rest and during exercise was consistently

higher in the Polynesian women than the Caucasian, although statistical significance was not quite reached. In the Caucasian group ^{13}C enrichment and RER were positively correlated at rest and after 10 minutes of exercise while these variables were uncorrected for the Polynesian women. As the simple sugar forms of carbohydrate are more enriched in ^{13}C than complex carbohydrate it was necessary that account be taken of simple sugar intake. With adjustment for diary intake of enrichment and RER is strengthened. Ethnicity was not a significant factor in predicting ^{13}C enrichment in expired breath. By either measure, RER or the adjusted ^{13}C , Polynesians did not burn proportionally more carbohydrate than Caucasians. The degree of central adiposity of all subjects (irrespective of ethnicity) measured as the logarithm of subscapular to triceps skinfold (Haffner et al., 1992) was a significant predictor of breath ^{13}C enrichment adjusted for enriched carbohydrate intake at rest and with continued exercise. Similarly, those with a greater percentage body fat tended to burn more carbohydrate. These two measures of fatness are interrelated and therefore were not included simultaneously in the same model. The higher R^2 values obtained for the prediction of breath ^{13}C enrichment after 15 minutes of exercise suggests that muscle glycogen better reflects long-term averaged dietary intake of enriched carbohydrate.

These results support the hypothesis that less enriched fuel (*i.e.*, lipid) is oxidised when RER is lower. Barstow et al. (1989) had put forward the same hypothesis but with the sample size in their study they were not able to show this relationship. Schoeller and colleagues (Schoeller et al., 1984) have presented evidence that resting breath ^{13}C is above to be predicted from the ^{13}C enrichments of plasma macronutrients - glucose, lipid and protein. However during the first 30 minutes of exercise their predicted value of ^{13}C enrichment computed from plasma metabolised suggested that more lipid was metabolised but the respiratory gas ratios measured at the same time, indicated increased carbohydrate oxidation. Romijn and colleagues (Romijn et al., 1992) have further confirmed that breath ^{13}C is a measure of substrate oxidation in stable states but found that 15 to 20 minutes were required to reach a plateau value of ^{13}C during exercise at 80-85% of

maximum oxygen uptake. As the exercise period and levels in the present study were less than this the ^{13}C changes cannot be related to absolute changes in substrate metabolism but the trend can be seen. It is interesting that the Polynesian group exhibit a small but statistically significant higher level of glucose in their blood in the fasting state, albeit within normal limits. Does this correlate with insulin resistance, greater glycogen stores, burning carbohydrate preferentially or another ethnic difference such as body fat distribution? In-depth studies measuring these variables more accurately under controlled conditions are needed to answer these questions.

Our study has shown that with the onset of exercise the natural enrichment of breath CO_2 falls within the first 6 minutes then has risen again 12 minutes after the start of exercise (Fig. 1). This phenomenon has also been shown by other groups (Schoeller et al., 1984; Barstow et al., 1989; Romijn et al., 1992; Wagenmakers et al., 1993) with the explanations independently offered by Schoeller and Barstow that changes in the size and dynamics of carbon dioxide stores are responsible. An extensive literature search has not been able to find any direct measures of the carbon isotope enrichment of glycogen and fat stores in the human. Schoeller (Schoeller et al., 1984) suggests that, as 60% of the resting bicarbonate pool is in skeletal muscle and the preferred pool is in skeletal muscle and the preferred metabolic of muscle is the isotopically light lipid, at the onset of exercise, increased blood flow to skeletal muscle will increase the fractional elimination rate of carbon dioxide from both these sources. It still remains possible that the change in ^{13}C enrichment at the onset of exercise may be due to a switch from liver glycogen which averages 70 g in an adult, to exercising muscle glycogen which is in the order of 200-300g as the major carbohydrate fuel, plus increased intramuscular lipid oxidation which is not reflected in the respiratory gas exchange as measured at the mouth. Romijn et al. (1992) fed enriched cornstarch and ^{13}C labelled glucose to glycogen-depleted subjects and calculated that during exercise 89% of the carbohydrate oxidised came from skeletal muscle glycogen. Gay and colleagues who fed subjects a diet high in naturally enriched carbohydrate (Gay et al., 1994) calculate that the

enrichment of the carbohydrate oxidised 25 minutes after the start of low intensity exercise (walking 4 km/hr and 10% slope) is approximately 30% lower than the enrichment of the carbohydrate oxidised at rest pointing to a shift in the stores of carbohydrate metabolised. Samples were not taken by these investigators between rest and 25 minutes so this change could have occurred before then.

The respiratory exchange ratio in this study was not adjusted for protein metabolism. As Garrow points out (Garrow, 1988) "the assumption that the quantity of carbon dioxide exhaled and the urea excreted relates to the same batch of fuel as that which the oxygen utilised is of doubtful validity". The bicarbonate and urea pools in the body are relatively large and the rate of excretion of these compounds does not necessarily match the rate of production, at least over times of less than an hour. Thus the measurement of the respiratory exchange ratio has inherent errors in assessing the metabolic fuel mix as does the ^{13}C enrichment. To reduce the error longer term measures of respiratory exchange ratio and the ^{13}C enrichment would need to be made and ideally would require the subject to be in a whole body calorimeter, with subject to be in a whole body calorimeter, with a controlled diet.

This study has shown that with exercise the enrichment of breath CO_2 is related to body fat percentage and distribution. Body fat distribution of the volunteers in this study is more central in the Polynesian women than Caucasian. The Polynesian women achieved higher exercise intensity at the first two levels of exercise than the Caucasian and this could be related to their higher fat-free mass. Intensity of exercise showed no significant difference when the influences of fatness and body size were examined. This may seem surprising in view of the potentially greater energy costs of moving greater body mass but the larger women did tend to walk at a slower rate and the gradient of the treadmill was adjusted to attain relatively the same increase in heart rate in all subjects. There are at least four possible explanations for the differences in breath ^{13}C enrichment between individuals and groups.

Firstly, there is interindividual variation of expired $^{13}\text{CO}_2/^{12}\text{CO}_2$ (Schoeller et al., 1980; Schoeller et al., 1984) which is probably caused

by the variation of the enrichment of the food eaten by each individual. Individual variation day to day averages about 5%. Differences in fasting RER (range 0.79 - 0.87, $n = 11$) between women on exactly the same diet have been measured (McNeill et al., 1988). Polynesian women reported a greater percentage of energy intake from fat and yet from their resting respiratory exchange ratio and the adjusted $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio appear to be burning the as carbohydrate/fat mix as the Caucasian women. Diet diary analysis validation by the doubly-labelled water method (Rush, 1997) showed no difference between the ethnic groups for the ratio of energy intake to total energy expenditure. Schutz and colleagues (Schutz et al., 1989) have shown under very controlled circumstances that increased dietary intake of fat fails to promote the oxidation of fat. This means that increased intake of fat is likely to result in increased storage of fat and to favour the development of obesity.

Secondly, if the various body stores of glycogen (liver and muscle) and triglycerides (intramuscular and adipose) differ in their enrichment values and size then any difference in the tissue source of oxidative substrate could result in differences in $^{13}\text{CO}_2$ not related to changes in RER (Barstow et al., 1989). Polynesian women had a higher fat-free mass than the Caucasian women in this study and therefore may have relatively larger glycogen stores.

Thirdly, the rate of change of RER with exercise is faster than the rate of change of $^{13}\text{CO}_2/^{12}\text{CO}_2$ enrichment. This is because carbon dioxide is stored in large quantities and between 3 and 5 different pools whose characteristics alter under different metabolic conditions (Barstow et al., 1988). The mean residence time for a carbon dioxide molecule in the body is between 60 and 90 minutes. Are the dynamics and size of these pools at rest and during exercise different between ethnic groups or associated with different body fat distribution?

Finally, the amount of carbohydrate oxidised could also be affected by sympathetic nervous system activity and the action of insulin. Increased levels of catecholamines increase mobilisation of glucose at rest and during exercise, particularly if the level of exercise is high. The resting blood glucose was higher in the Polynesian women

in this study, and whilst we did not measure fasting insulin it is likely that this was higher in the Polynesian women also (personal communication, D. Simmons, 1997). In a study of 720 Caucasian men and women, Nagy et al. (1996) have shown that fasting insulin is inversely related to fat oxidation.

The thrifty genotype hypothesis that obesity cannot always be attributed to gluttony and sloth means that obesity should be considered as resulting, at least in part, from factors associated with metabolism substrate availability and genetics. ^{13}C enrichment of expired breath, adjusted for enriched sugar intake, does tend to be less with the lower RER values, which adds strength to the hypothesis that the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio in expired breath is a measure of the fuel mix metabolised. Analysis of the diet diaries indicated similar food choices across the two ethnic groups (Gonelevu et al., 1997). It is purported that an increased intake of fat does not suppress appetite yet it is the most energy rich of the four major nutrients. From our data it appears that the higher the percentage of fat in the diet the more likely that there will be increased consumption of total energy. This is particularly true of the Polynesian group and although higher intakes may be due to a greater honesty in reporting dietary intake of fat it still means that Polynesians who eat more fat are more likely to gain weight because of the greater energy intake. The hypothesis that fat is stored as fat, whilst carbohydrate is more readily metabolised is supported by a number of investigators whose publications have been reviewed separately by Westerterp and Schutz (Schutz, 1993; Schutz, 1995; Westerterp et al., 1995). Given that the reported diet of the subjects in this study had an average fat content of more than 35%, and the intake of fatty, fast food (e.g. McDonald's, Kentucky Fried Chicken, and fish and chips) was on average 2 to 3 times per week it is to be expected that this obesogenic environment linked with a tendency to metabolise carbohydrate rather than fat should result in subsequent weight gain. Longitudinal studies would be needed to confirm this.

In summary, this study supports the contention of a different metabolism of carbohydrate and fat in women with more central body fat and more fat. Apparent ethnic differences were accounted for by differences in the diet (sugar),

percentage body fat and distribution of body fat. Body fat is more centrally distributed in the Polynesian women. A low ratio of fat to carbohydrate oxidation, and higher energy and fat intake all could contribute to weight gain over a period of time. The evidence provided here does support the convention that women with more fat (both percentage fat and absolute fat mass) and with more fat in the central body area may burn relatively more carbohydrate than the leaner subjects with relatively more peripheral fat. It is interesting that the reported sugar intake correlates well with the enrichment in the breath for both groups yet the Polynesian group report that they eat more fat but cannot be shown to burn more fat. Longitudinal studies of Polynesian and Caucasian men and women over a wider age range and with more precise measurement of dietary intake and body composition are needed to explore these relationships further.

REFERENCES

- Acheson, K.J., Schutz, Y., Bessard, T., Flatt, J.-P. and Jequier, E.: Carbohydrate metabolism and de novo lipogenesis in human obesity. *Am. J. Clin. Nutr.*, **45**: 78-85 (1987).
- Barstow, T., Cooper, D., Epstein, S. and Wasserman, K.: Changes in breath $^{13}\text{CO}_2/^{12}\text{CO}_2$ consequent to exercise and hypoxia. *J. Appl. Physiol.*, **66**(2): 936-942 (1989).
- Barstow, T., Cooper, D., Springer, C. and Epstein, S.: Estimation of CO_2 stores by tracer dilution and VCO_2 kinetics. *Federation of American Societies for Experimental Biology Journal (FASEB Journal)*, **2**: A306 (1988).
- Black, A.E., Prentice, A.M., and Coward, W.A.: Use of food quotients to predict respiratory quotients for the doubly-labelled water method of measuring energy expenditure. *Hum. Nutr. Clin. Nutr.*, **40C**: 381-391 (1986).
- Burlingame, B.A., Milligan, G.C., Apimerika, D.E. and Arthur, J.M.: *The concise New Zealand food composition tables*. Palmerston North, New Zealand Institute for Crop and Food Research. New Zealand Public Health Commission (1994).
- Coward, W.A., Ritz, P. and Cole, T.J.: Revision of calculations in the doubly labeled water method for measurement of energy expenditure in humans. *Am. J. Physiol.*, **267**: E805-807 (1994).
- Dowse, G., Zimmet, P., Collins, V. and Finch, C.: Obesity in pacific populations. In: *Obesity*. P. Bjorntorp and B. Brodoff (Eds.). Philadelphia. JB Lippincott Co: 619-639 (1992).
- Durnin, J. V.G. A. and Womersley, J.: Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br. J. Nutr.*, **32**: 77-97 (1974).
- Ferrannini, E.: The theoretical bases of indirect calorimetry: a review. *Metabolism*, **37** (3): 287-301 (1988).
- Flatt, J.P., Ravussin, E., Acheson, K.J. and Jequier, E.:

- Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. *J. Clin. Invest.* 76 : 1019-1024 (1985).
- Garrow, J.S. : *Obesity and Related Diseases*. Churchill Livingstone, Edinburgh (1988).
- Gay, L.-J., Schutz, Y., Die Vetta, V., Schmeiter, P., Tappy, L. and Jequier, E. : Measurement of $^{13}\text{CO}_2$ in expired air as an index of compliance to a high carbohydrate diet naturally enriched in ^{13}C . *Int. J. Obesity*, 18 : 591-595 (1994).
- Gonelevu, S., Rush, E. and Lau, M. : Fruit, vegetable and cereal intake of Polynesian and European women in Auckland. *Pacific Health Dialog*, 4 (2) : 11-19 (1997).
- Haffner, S.M., Valdez, R.A., Hazuda, H.P. : Prospective analysis of the insulin-resistance syndrome (syndrome X). *Diabetes*, 41 : 715-722 (1992).
- Lange-Anderson, L., Shephard, R., Denolin, H., Varnauskas, E. and Masironi, R. : *Fundamentals of exercise testing*. World Health Organisation, Geneva (1971).
- Mackerras, D. : *Interpreting dietary data*. Hillgate Hill, Queensland, Xyris Software (1991).
- McGarvey, S.T. : Obesity in Samoans and a perspective on its etiology in Polynesians. *Am. J. Clin. Nutr.*, 53 (Suppl) : 1586S-1594S (1991).
- McNeill, G., Bruce, A.C., Ralph, A. and James, W.P.T. : Inter-individual differences in fasting nutrient oxidation and the influence of diet composition. *Int. J. Obesity*, 12 : 455-463 (1988).
- Nagy, T., Goran, M., Weinsier, R., Toth, M., Schutz, Y. and Poehlman, E. : Determinants of basal fat oxidation in healthy Caucasians. *J. Appl. Physiol.*, 80 : 1743-1748 (1996).
- Neel, J.V. : A "thrifty" genotype rendered detrimental by "progress"? *Am. J. Hum. Genet.*, 14 : 353-362 (1962).
- Ravussin, E. : Low resting metabolic rate as a risk factor for weight gain : role of the sympathetic nervous system. *Int. J. Obesity*, 19 (Suppl 7) : 58-59 (1995).
- Romijn, J., Coyle, E., Hibbert, J. and Wolfe, R. : Comparison of indirect calorimetry and a new breath $^{13}\text{C}/^{12}\text{C}$ ratio method during strenuous exercise. *Am. J. Physiol.*, 263 (26) : E64-E71 (1992).
- Rush, E. : *Energy Expenditure and Metabolism in Caucasian and Polynesian Women*. Ph. D thesis. University of Auckland : 191 pages (1997).
- Schmidt, H.-L. and Metges C. : Variations of the Natural isotope abundance in diet-causes of artifacts or the base of new possibilities in stable isotope tracer work? *Clinical Nutrition and Metabolic Research Proc.* 7th Congr. ESPEN, Munich, Karger, Basel (1986).
- Schoeller, D.A., Brown, C., Nakamura, K., Nakagawa, A., Mazzeo, R.S., Brooks, G.A. and Budinger, T.F. : Influence of metabolic fuel on the $^{13}\text{C}/^{12}\text{C}$ Ratio of breath CO_2 . *Biomed Mass Spectrom.* 11 (11) : 557-561 (1984).
- Schoeller, D.A., Klein, P.D., Watkins, J.B., Heim, T. and MacLean, W.C. : ^{13}C abundances of nutrients and the effect of variations in ^{13}C isotopic abundances of test meals formulated for $^{13}\text{CO}_2$ breath tests. *Am. J. Clin. Nutr.*, 33 : 2375-2385 (1980).
- Schutz, Y. : The adjustment of energy expenditure and oxidation to energy intake : the role of carbohydrate and fat balance. *Int. J. Obesity*, 17 (Suppl 3) : S23-S27 (1993).
- Schutz, Y. : Abnormalities of fuel utilisation as predisposing to the development of obesity in humans. *Obesity Research* 3 (Suppl 2) : 173s-178s (1995).
- Schutz, Y., Flatt, J.P. and Jequier, E. : Failure of dietary fat intake to promote fat oxidation : a factor favouring the development of obesity. *Am. J. Clin. Nutr.*, 50 : 307-314 (1989).
- Shephard, R.J. : *Physiology and Biochemistry of Exercise*. Praeger Publishers, New York (1982).
- Wade, A.J., Marbut, M.M. and Round, J.M. : Muscle fibre type and aetiology of obesity. *Lancet*, 335 : 805-808 (1990).
- Wagenmakers, A.J.M., Rehrer, N.J., Brouns, F., Saris, W.H.M. and Halliday, D. : Breath $^{13}\text{CO}_2$ background enrichment during exercise : diet related differences between Europe and America. *J. Appl. Physiol.*, 74 (5) : 2353-2357 (1993).
- Westerterp, K.L., Wouters, L. and Lichtenbelt, W.D.v. : The Maastricht Protocol for the measurement of body composition and energy expenditure with labeled water. *Obes. Res.*, 3 (Suppl 1) : 49-57 (1995).
- Wilmore, J.H. and Costill, D.L. : *Physiology of Sport and Exercise*. Human Kinetics, Champaign, IL (1994).