ADAPTIVE CHANGES IN BASAL METABOLIC RATE AND THERMOGENESIS IN CHRONIC UNDERNUTRITION

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Abstract

Metabolic adaptation during chronic undernutrition represents a complex integration of several processes which affect the total energy expenditure of the individual. Basal metabolic rate (BMR) is reduced; reductions in BMR per unit fat free mass (FFM) is difficult to demonstrate. BMR changes in undernutrition reflect the low body weight as well as alterations in the composition of the FFM; more specifically changes in the ratio of viscera to muscle compartments of the FFM. Thermogenic responses to norepinephrine are transiently suppressed but recover rapidly on repeated stimulation. Dietary thermogenesis is enhanced possibly the result of increases in tissue synthesis within the body. Changes in BMR and thermogenesis suggestive of an increase in metabolic efficiency is thus difficult to demonstrate in chronic undernutrition.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

The Project under review supported by the Co-ordinated Research Programme (CRP) on the "Application of stable isotope tracers in human nutrition research" is a joint initiative of the IDECG and CREAM aimed at the evaluating the role of metabolic adaptation to variations in energy intake. That individuals adapt to variations in intake is well recognised [1]. There are however, several interesting aspects to the question of metabolic adaptation both from a purely scientific point of view as well as from the view point of estimating the energy requirements of individuals. To quote James [2], "the new approach to energy requirements established by an Expert group [3] has implied that energy metabolism does not readily adapt in man without concordant and potentially deleterious changes in body weight and spontaneous physical activity". Sukhatme & Margen have challenged this on statistical grounds so successfully that in the Fifth World Food survey [4] there was a special adjustment in the calculation of national energy needs which reduced the maintenance energy requirement value to only 1.2 times the estimated basal metabolic needs of the population rather than 1.4 or more times the basal metabolic rate (BMR) as suggested by the Expert group. The choice of the lower value as cut-off point below which a population group was classified as malnourished had the effect of reducing substantially the purported numbers of malnourished in the world. The choice of appropriate energy requirement values for people working and living normally clearly depends on resolving this issue and an independent measure of the energy needs of desirable spontaneous physical activity in free-living subjects was badly needed. The doubly-labelled water (DLW) technique was ideally suited to fill this lacunae. A

Consultative Group of the ACC Sub-Committee on Nutrition of the United Nations has made a thorough examination of the Sukhatme - Margen hypothesis and proposed a number of protocols of increasing complexity in an attempt to test the hypothesis. The protocols envisaged studying human subjects before, during and after a sustained perturbation in their energy intake by 25 percent in either direction while estimating the components of energy expenditure and body composition using both indirect calorimetry and stable isotopic tracer techniques. As a part of this co-ordinated approach to resolving this vexed question, it was proposed to study groups of individuals using indirect calorimetry and the doubly labelled water technique while being maintained on plus 25 percent of their energy intakes during a 12 week period. It was envisaged that financial support would be forthcoming for the study from a funding agency while the required stable isotopes for the study would be made available by this Co-ordinated Research programme under this research contract.

During the period under review, substantial progress has been made in the conduct of this investigation:

(1) Orders were placed for the purchase of DLW soon after the initiation of the project under the CRP. However, due to the considerable demand for the isotope and the world-wide shortage of ^{18}O in the market there were delays in the supply of DLW. Orders placed by the Rowett Research Institute, Aberdeen on our behalf have been complied to and the isotope has been delivered.

(2) The original arrangement of the joint, multi-country project under this programme on energy adaptation in man under the initiative of CREAM was that isotopic analysis for all participants under this joint study was to be undertaken at the Rowett Research Institute, Aberdeen. With the Department of Science and Technology (DST), Ministry of Science and Technology, Govt. of India, taking a decision to establish a Stable Isotope facility for human nutritional studies at the Nutrition Research Centre, Department of Physiology, St. John's Medical College, Bangalore under the Intensification of Research in High Priority Areas (IRHPA) scheme, it was decided that the analysis of tracer samples for this study will be made at Bangalore. It was hoped that this arrangement would provide sufficient experience and technical competence to the analytical facilities being set up here. Comparisons of analysis of some of the samples in two laboratories would also ensure quality control and reliability. With the grant made available by the DST, it was decided to acquire two independent isotope ratio mass spectrometer (IRMS) systems; one with a dual inlet dedicated to deuterium analysis using the standard zinc reduction method and the other for the analysis of ^{18}O, ^{13}C and ^{15}N. The two systems have been acquired with all the necessary accessories from Europa Scientific, Crewe, UK and have been installed and have been operational since July 1991.

2. EXPERIMENTAL METHODS

During the period under Report, several preliminary studies and analysis have been carried out. They are:

(1) A comparison of Food Quotients (FQ) and Respiratory Quotients (RQ) in well-nourished controls and undernourished or chronically energy deficient (CED) subjects for deriving energy expenditure from CO_2 production rates using the DLW technique.

(2) A study on the effects of short-term supplementation on total energy expenditure (TEE) and Respiratory Quotients (RQ) of undernourished subjects.

(3) A study of the rate of incorporation of deuterium into lipids in normal subjects and during positive energy balance in chronically energy deficient subjects.

3. RESULTS

Food quotients versus respiratory quotients for the calculation of energy expenditure measured by DLW technique.

The DLW technique estimates the rate of production of CO_2 rather than O_2 consumption. This is a disadvantage since the energy equivalent of CO_2 varies to a greater extent that the energy equivalent of O_2. The variation is determined by the substrate being oxidised which is indicated by the RQ. Since mean RQs of free-living subjects are not readily and continuously available, the earlier studies using DLW technique assumed an average value for RQ of 0.85 which was an approximation at best. Black and her colleagues [1986] suggested a more elegant approach for this by attempting to predict the RQ from the macro nutrient content of the subject's diet. This was termed the food quotient or FQ.

Under conditions of energy balance the FQ must equal the RQ. This may not be true of conditions associated with energy imbalance as in the case of refeeding undernourished subjects. This is a crucial factor which can contribute to errors. Black et al. [5] have suggested methods for converting FQs into RQs under conditions of energy imbalance in adults. An additional problem is that of sequestration of the isotope which is likely to occur in any condition associated with positive energy balance. Isotope sequestration is an important issue that will be addressed later by the studies being conducted in well nourished and CED subjects.

Comparisons of FQs calculated by methods suggested by Black et al. [5] and RQs actually measured in studies have been done by us under different experimental conditions in both well-nourished and CED subjects.

3.1. Comparisons of Food Quotients of weighed intakes with mean 24-hour Respiratory Quotients by indirect calorimetry.

The mean RQs obtained during a 24 hour-cycle in an indirect calorimeter were computed in both well-nourished controls and undernourished CED subjects while in the whole body calorimeter (Table 1).

Differences between RQs and FQs were seen in the undernourished subjects only, with RQs being higher than FQs. The FQs were obtained from the composition of the actual food provided to the subjects during 36 hour calorimetry runs which are likely to be different from their habitual diets in terms of their macronutrient composition.

3.2. Comparisons of Food Quotients from dietary recall with post-absorptive Respiratory Quotients.

FQs calculated from dietary recall of 24 hour intakes over several days using the formula suggested by Black et al. [5] were made in a number of well-nourished and CED subjects and were compared with the 12-14 hour post-absorptive, early morning RQs obtained during the measurement of a basal metabolic rate (BMR). No differences were seen between the FQs and early morning, fasted RQs in either group of subjects (Table 2).

As reported several times in the past [6,7] the fasting RQs of the undernourished were significantly higher than those of the well-nourished and their FQs also corroborated this, since the latter were habituated to a high carbohydrate diet.

3.3. Comparisons of Food Quotients from weighed intakes with post-absorptive Respiratory Quotients.

Comparisons of FQs from weighed intakes over several days with the RQs of the same subjects obtained during a standard BMR measurement showed FQs were lower than RQs (Table 3) and that the difference was statistically significant.

3.4. Comparison of Food Quotients and Respiratory Quotients before and after supplementation in undernourished subjects.

The most significant differences in FQs and RQs were seen when comparisons of 24 hour dietary recall were made in undernourished subjects during a 12 week refeeding study which included measurements made during refeeding with the supplementation diet. The mean 24 hour RQ while in a calorimeter was 1.02 while the FQs were comparable with the intakes and hence much lower (Table 4).

The differences in FQs calculated from both weighed intake and 24 hour dietary recall were significantly different from the measured RQs. These undernourished subjects who were gaining weight with supplementation had very different mean RQs and FQs. These observations raise important technical problems related to the use of FQ values in the conversion of the rates of CO_2 production to total energy expenditure in conditions of energy imbalance. This has considerable relevance to the study on metabolic adaptation which involves underfeeding or overfeeding by at least 25 percent intake for a period of 12 weeks as per the ACC/SCN protocols. Attempts have been made to address these issues. The results may have an important bearing on the increasing use of DLW techniques in population groups world-wide.

3.5. An evaluation of the errors associated with the use of CO_2 production rates alone when calculating energy expenditure.

The use of the DLW technique results in estimation of total energy expenditure (TEE) of human subjects from measures of CO_2 production rates alone i.e., without measuring O_2 consumption of the subject. In this situation:

TEE = CO_2 production (litre) * Energy equivalent of CO_2 (kJ per litre)

The energy equivalent of 1 litre of CO_2 (E eq CO_2) varies considerably more than that of O_2 (E eq O_2). Hence in situations where CO_2 alone is used to predict TEE, it is essential that an appropriate E eq CO_2 for the body is assumed. Since E eq CO_2 and E eq O_2 of the individual fuels of the body such as carbohydrates, proteins, and fats or their mixtures are related to the RQ, the RQ can be used to obtain an approximate estimate of E eq CO_2 and E eq O_2. The problem of course is to obtain a representative RQ or FQ which would be truly indicative of the E eq CO_2 of the body. The E eq CO_2 of the human diet may vary from 21.8 to 24.2 kJ per litre with an FQ range of 0.83 to 0.95, a variability confirmed by dietary intake studies in over 147 countries [8].

When the body is in nutrient and energy balance, then:

RQ = FQ and E eq CO_2 of the body = E eq CO_2 of the diet.

This may not however apply to situations where energy imbalance exists. During positive energy balance as during overfeeding when excess energy is largely being deposited as fat, then:
RQ > FQ and E eq CO_2 of the body < E eq CO_2 of the diet.

During negative energy balance as in situations associated with body weight loss, the deficit of energy will be made up largely by the oxidation of endogenous fat, and then:
RQ < FQ and E eq CO_2 of the body > E eq CO_2 of the diet.

Hence RQ and FQ values can introduce errors in the estimation of TEE in situations of energy or nutrient imbalances. Diets with low E eq CO_2 and correspondingly high FQs will have a more pronounced effect on the E eq CO_2 of the body for a given degree of energy imbalance [8]. The degree of energy imbalance has to be however fairly large to produce substantial errors in the E eq CO_2 of the body. Underfeeding or overfeeding to the extent of 50% of TEE will alter E eq CO_2 of the body and its RQ by 4 to 11% depending on the composition of the diet. Such a degree of imbalance over a period of 2 weeks i.e. the usual duration of a DLW study may be detected as body weight changes in the subjects and these can be used to correct for the errors. It is however important to remember that RQ and FQ values used in the conversion of CO_2 production rates to estimates of TEE using energy equivalents of CO_2 can account for a substantial proportion of the errors associated with this method when used in free living subjects who are not in zero energy balance.

3.6. Short-term energy supplementation of undernourished or chronically energy deficient adult subjects.

This study involved measurements of body composition and 24 hour energy expenditure measurements by whole body indirect calorimetry in 9 chronically energy deficient adult males. The measurements were made prior to, during, and following 12 weeks of an additional supplement to the habitual diet. The supplement consisted of two snacks being provided for a period of 23 weeks equivalent to 800 kCal energy and 15 gm protein every day. The chronically undernourished subjects were physically active, non-anaemic adults aged between 18 to 28 years. They were from a poor socio-economic background resident in a nearby urban slum. The mean height of the subjects was 165 cm, mean body weight approximately 45 kg and mean body mass index (BMIs) were < 18.5. Body composition was estimated by densitometry and by anthropometry while 24 hour energy expenditure (TEE) and basal metabolic rates (BMR) were assessed by indirect whole body calorimetry [9]. In addition, both non-shivering thermogenesis in response to intravenous norepinephrine infusion and diet induced thermogenesis following a standard liquid meal stimulus were assessed prior to and after the 12 weeks of supplementation. BMR measurements were made serially throughout the study along with measures of changes in body composition.

The results of this refeeding study of undernourished subjects showed that energy supplementation produced an increase in body weight, in fat-free mass (FFM) and in fat content (Table 5). The results of the serial changes in body composition during supplementation indicate that the total increase in body weight occurred over the entire 12 week period, 72% of the increase occurred within the first 3 weeks of feeding, with almost equal proportions of fat and FFM being gained. The RQs seen during the BMR measurements made serially during this period showed a significant increase, with RQ values > 1.0 from the third week onward [10]. It is well recognised that extra carbohydrate provided in the diet of animals results in greater amounts of carbohydrate oxidation in the fasted state [11]. Human studies have also shown that an excess of carbohydrate consumed (i.e. 1500 kCal per day over 7 days) resulted in RQs of 1.5 which were associated with an increase in BMR possibly due to an increase in the costs of *de novo* lipogenesis [12]. Fasting substrate oxidation rates calculated in our refeeding studies also support the conclusion that possibly *de novo* lipogenesis as well as protein synthesis was occurring in our subjects with the use of carbohydrate as the predominant fuel being oxidised. The data also indicate that body fat constitutes a greater proportion of the weight gained (72%) from the third week of supplementation onwards, while at the same time the RQs rise above 1.0. Once body weight gains level off, about the ninth week of supplementation and no further substantial gain in body fat occurs, then the RQs tend to return to basal levels. The percent increase in body fat content compared to pre-supplementation levels was 21.2 %, 21.7 %, 21.3 % and 19.7 % during the 3rd, 6th, 9th and 12th weeks of supplementation respectively.

BMRs showed an increase [10] as the supplementation proceeded (Table 5). However, no differences were observed between pre-supplementation and the 12 week post supplementation measurements of thermogenic responses to norepinephrine [13] and dietary stimulus [14] in these subjects.

The findings of this study have implications to the possible interpretation of data using DLW techniques in human subjects during states of energy imbalance. Haggarty

[15] has analyzed the consequences of isotope sequestration and the subsequent exchange of the sequestered isotope with the body water pool in the interpretation of the DLW technique. The process of lipogenesis from 2 carbon units provides a route for the entry of water hydrogen into the stable carbon-hydrogen bonds of the fatty acid molecule. It has been estimated that about 45% of hydrogen atoms of newly synthesised fatty acids are derived from body water [16]. Haggarty [17] has suggested that for deuterium the sequestration value for fat should be 53%; i.e. 53% of the hydrogen of newly synthesised fatty acid will be derived from water labelled with deuterium. He has also summarised the data indicating that sequestration may be occurring in human subjects also during *de novo* lipogenesis. Although it is assumed that this may be a relatively small effect, it cannot be dismissed readily under the following two experimental situations:

(1) when designing studies which are likely to induce conditions of energy imbalance such as the present study which produced body weight gain, more specifically an increase in body fat resulting in possible sequestration of isotope;

(2) when the DLW water technique is used to estimate energy expenditure in conditions of energy imbalance, as in the present study, since the sequestration process is likely to underestimate the rate of production of CO_2 and will add to the errors associated with this technique.

Three important issues have been raised thus far in this report:

(1) the errors associated with the use of RQ or FQ in the conversion of rates of CO_2 production to estimates of energy expenditure;

(2) the errors associated with the use of CO_2 production rates alone in arriving at estimates of energy expenditure;

(3) the errors that may underestimate CO_2 production rates in situations where sequestration of the isotope is likely to occur.

All three issues may indeed be limitations of this isotopic tracer method particularly in conditions of energy imbalance. This needs to be recognised and dealt with. A recent study [18] conducted in 6 subjects, half of whom were overfed and the other half partially energy restricted, showed that the estimated rate of CO_2 production by the DLW technique was under estimated by 17% 32.4%, 13.5% and 10.2% in 4 of the 6 subjects when compared with whole body indirect calorimetry over a period of 12 days. The contribution from a predicted maximum effect of sequestration due to nutrient deposition in the 3 subjects who were overfed accounted for only between 4.6% to 6.7% of the underestimate of CO_2 production. It is likely that isotopic variations in the background may have contributed to the rest of the error.

These issues have enormous implications to studies that have been suggested and protocols developed by the ACC/SCN Sub-Committee since the objective of these studies is to look for metabolic adaptation. The studies are designed to look for small increases in metabolic efficiency. The small errors in the DLW method in particular may be crucial and need to be considered seriously before arriving at far reaching conclusions when the tracer techniques are used in these protocols.

3.7. Sequestration of deuterium into lipids in normal subjects and during positive energy balance by refeeding in chronically energy deficient subjects.

The validity of the DLW technique depends on a number of assumptions one of which is that there is no loss of either of the isotopes i.e., ^2H or ^{18}O, as products other than H_2O or CO_2. The DLW method thus assumes that both tracers are lost only from the body water pool either in water or as CO_2 and hence any other transformation in the body involving these isotopes will introduce an error into the calculations of the rate of production of CO_2. If deuterium was lost by any other route other than water this would cause an over-estimation of water flux and the difference between the deuterium flux rate and the oxygen- 18 flux rate, i.e., the rate of production of CO_2, would be under estimated. Deuterium incorporation into body solids during reductive biosynthesis can cause such as error in the estimation of CO_2 production rate. From calculations it has been estimated that the greatest potential for isotopic sequestration occurs in fat followed by carbohydrate and lastly protein. Of these, fat is the only one which could significantly affect the accuracy of the estimates of CO_2 production rate while using the DLW technique.

In a collaborative study being carried out by the Rowett Research Institute, Aberdeen and the Nutrition Research Centre, Bangalore, the extent of sequestration of deuterium is being investigated in both weight-stable well-nourished adults and in chronically undernourished subjects while they are being supplemented and hence in positive energy balance. The experimental protocol is illustrated graphically in Fig 1.

Figure 1 represents the phase I of the study which lasted for 4 weeks. On day 0, subjects were given an infusion of norepinephrine to stimulate lipolysis and a 200 ml blood sample was obtained for the extraction and analysis of blood lipids. They were then given deuterium to raise their body water enrichment by 500 ppm over the normal background enrichment of about 150 ppm. In the first two weeks, the subjects were given enough deuterated water each day to maintain their body water enrichment at 500 ppm excess. At the end of this 2 week period, the norepinephrine adminstration and the blood sampling protocol was repeated. Deuterium estimations of urine and blood were carried out using the IRMS acquired by the Nutrition Research Centre, Bangalore recently, while the plasma extracted from the blood samples obtained before and after the daily administration of deuterium was transferred to the Rowett Research Institute, Aberdeen, for the purpose of analysis. The plasma samples would be used to separate lipid; both VLDL and FFA and the extent of ^2H incorporation into these lipids will be measured.

The experiments have been carried out in 9 well-nourished young adults. In each group, only 6 subjects have been dosed with ^2H_2O and their bloods collected following intravenous norepinephrine infusion on two occasions. The other 3 subjects were not dosed but provided daily and weekly urine samples for monitoring the changes in background enrichment over the duration of the study. The results of the deuterium analysis during this study in both the subjects who were dosed and controls is shown in Table 6 and also diagrammatically represented in Fig 2.

During phase II of the study, the subjects continued to collect weekly spot urines while on normal water intakes for up to 16 - 20 weeks after the initial dosing. The deuterium concentrations were estimated at Bangalore. The results of the deuterium concentration are presented in Table 7 and are being sequentially analyzed at the Rowett to look for recycling of the sequestered deuterium which is likely to maintain the enrichment of the isotope over and above the background levels around 12 - 16 weeks after the initial dosing.

4. CONCLUSIONS

The studies carried out under the CRP programme of the IAEA have led us to conclude the following:

(1) Food Quotients do not always reflect the Respiratory Quotients and hence may affect the estimates of total energy expenditure made using the DLW technique.

(2) Energy imbalance following increases in energy intake by supplementation of food affects the Respiratory Quotients and hence estimates of energy expenditure and

(3) Sequestration of the isotope during periods of supplementation may influence the estimates of energy expenditure during periods of energy imbalance in human subjects.

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TABLE I. MEAN 24 hr RQ FROM INDIRECT CALORIMETRY vs FQ FROM WEIGHED FOOD INTAKE

Subjects	RQ	FQ	P Value
Well-nourished	0.83	0.82	NS
Under-nourished	0.93	0.90*	$P<0.01$

TABLE II. RQ FROM BASAL METABOLIC RATE vs FQ FROM DIETARY RECALL

Subjects	RQ	FQ	P Value
Well-nourished	0.86	0.84	NS
Under-nourished	0.95	0.94	NS

TABLE III. RQ FROM BASAL METABOLIC RATE vs FQ FROM WEIGHED INTAKE

Subjects	RQ	FQ	P Value
Well-nourished	0.85	0.81	$P<0.02$
Under-nourished	0.93	0.88	$P<0.05$

TABLE IV. RQs and FQs BEFORE AND AFTER SUPPLEMENTATION

Pre-supplementation		Post-supplementation		
RQ	FQ	RQ	FQ	
	(dietary recall)		(dietary recall)	(weighed intake)
0.95	0.92	1.02	0.91*	0.92*

TABLE V: BODY COMPOSITION AND BASAL METABOLIC RATES OF SEVEN CHRONICALLY UNDERNOURISHED SUBJECTS, BEFORE AND DURING SUPPLEMENTATION

	Pre-supplementation	DURING SUPPLEMENTATION (WEEKS)			
		3rd	6th	9th	12th
Body weight (kg)	43.5 (0.8)	44.8 (1.0)‡	45.4 (1.0)‡	45.3 (0.9)	45.3 (1.1)
Fat (kg)	4.8 (0.3)	5.4 (0.4)*	5.9 (0.4)†	5.9 (0.5)	5.8 (0.6)
FFAA (kg)	38.7 (0.8)	39.4 (0.9)†	39.5 (0.8)*	39.4 (0.7)	39.5 (0.9)
Corrected arm muscle area(cm2)	28.6 (1.1)	29.3 (0.9)	29.8 (1.0)	29.8 (1.3)	29.3 (1.2)
RQ	0.97 (0.03)	1.08 (0.04)	1.17 (0.06)*	1.13 (0.04)	0.98 (0.04)*
BMR (MJ/d)	4.81 (0.11)	5.21 (0.10)‡	5.43 (0.10)†	5.65 (0.08)†	5.89 (0.28)*
BMR.WT-1 (kJ/kg/d)	110.5 (2.5)	116.4 (2.4)*	119.7 (3.0)*	125.0 (3.0)‡	129.8 (5.4)*
BMR.FFM-1 (kJ/kg/d)	124.3 (2.3)	132.3 (2.2)†	137.5 (2.9)‡	143.4 (2.4)†	148.5 (5.2)

Values are Mean (SEM); Overall significance assessed using an ANOVA for repeated measures

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.005$;

TABLE VI: DEUTERIUM LEVELS (in ppm) IN SEQUESTRATION STUDY

Subject	Predose	Post dose			
	(urine)	4 hr (plasma)	day 1-14 (urine)	day 21 (urine)	day 28 (urine)
EOO1	153.7916 (± 1.0246)	671.3236 (±0.9588)	706.5119 (± 1.0085)	433.9322 (± 1.3101)	284.7037 (±0.6849)
E002	153.6221 (±0.7561	721.9221 * (±6.8150)	770.4349 (± 1.7132)	520.3071 (± 1.0747)	333.6667 (±0.9048)
E003	152.868 (±0.8998)	657.4569 (±0.9608)	703.1458 (±0.8192)	507.2196 (±2.7415)	347.4060 (±2.1524)
E004	154.2716 (±0.9948)	707.1252 (±2.2626)	651.3972* (±4.6728)	329.3466 (±2.4551)	223.4970 (±0.772)
E005	153.8188 (±0.4974)	727.2550 (± 1.8894)	756.0982 (± 1.9157)	542.7584 (±0.8582)	360.4940 (± 1.0891
E006	153.9292 (±0.6083)	646.7977 (±2.4627)	548.6115 (±0.5595)	260.8186 (±0.8477)	183.6070 (±0.021)
C-1	154.3946 (±0.6234)	----	153.9221 (±0.8659)	156.1202 (±0.3494)	156.1310 (±0.179)
C-2	154.1734 (±0.1343)	----	152.4202 (±0.2839)	154.3797 (±0.3093)	154.5120 (±0.0685)
C-3	156.1779 (±0.1290)	----	156.1009 (±0.3657)	156.1255 (±0.0061	156.9517 (±0.1791

Dose administered 19.076 g of 99.9% D2O per 50 kg body weight
Maintenance dose: = 1.813 g of 99.9% D2O per 50 kg body weight
Mean (± SD)   * need to be repeated

TABLE VII: LEVELS OF DEUTERIUM (in ppm) IN BODY FLUIDS (URINE) OVER WEEKS AFTER INITIAL DOSING

SUBJECTS: / WEEKS	A	Sh	I	Su	An	C
5	216.4015	250.4226	-	176.6926	-	163.8009
6	190.8589	-	-	161.8700	213.8543	157.6332
7	169.2429	188.6395	186.9018	160.6145	184.3615	-
8	144.9610	-	-	-	176.6202	155.1570
9	-	-	162.2289	156.3120	163.8405	151.5014
10	152.4005	162.5526	157.6199	156.1252	160.0087	154.7099
11	153.8742	161.1819	158.3765	-	156.5932	152.3509
12	154.4566	159.4369	163.9424	-	156.1667	-
13	154.3791	156.2841	157.4663	-	-	-
14	154.3983	156.4053	155.6120	-	164.3977	155.1853
15	155.2922	158.1958	-	155.1888	164.7593	155.7192
16	154.8292	157.0106	155.3904	155.0188	154.7702	156.0628
17	-	157.2381	-	154.5588	154.8432	-
18	156.4187	155.3265	154.4154	-	153.2937	156.9100

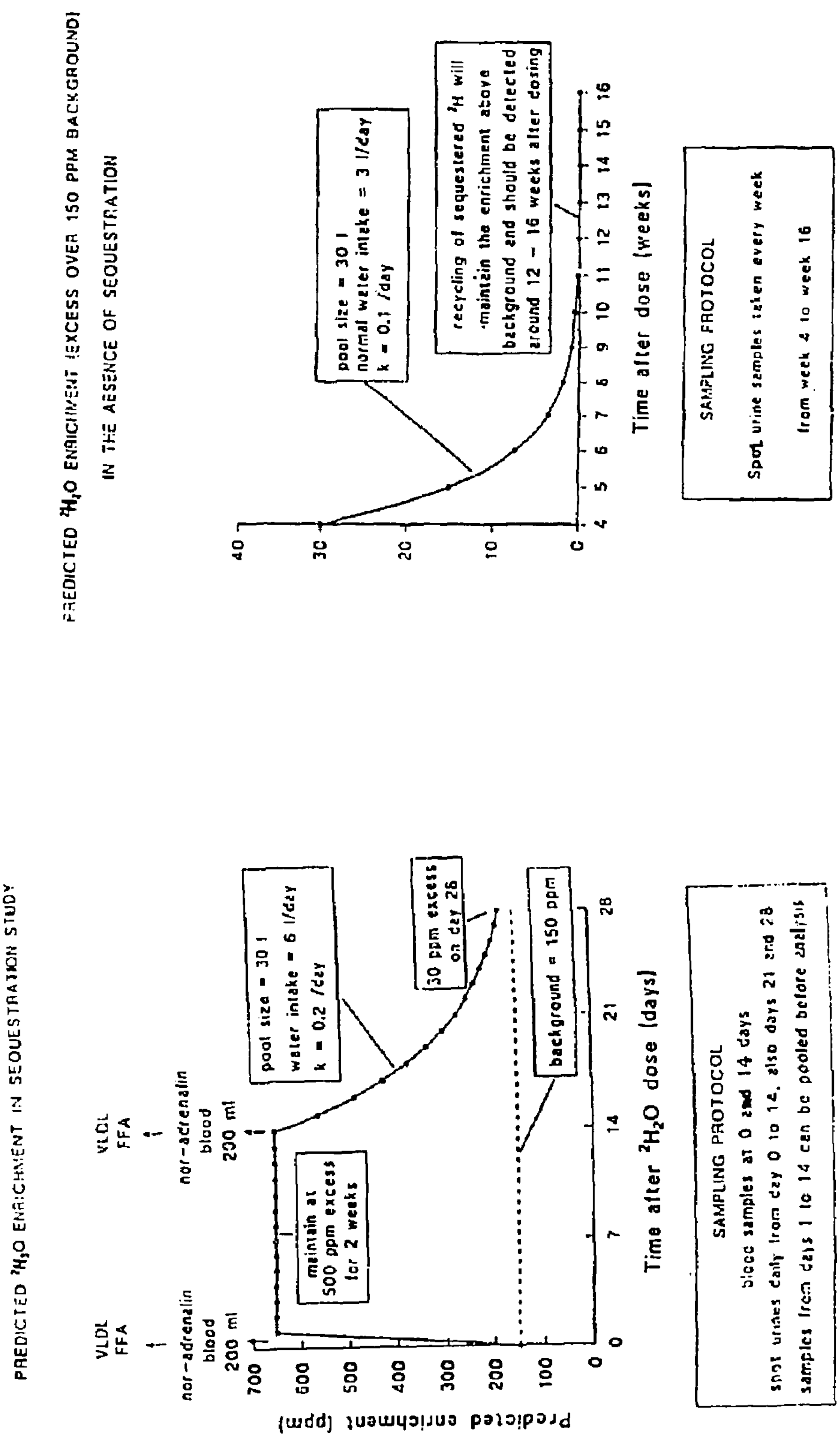
PREDICTED ²H₂O ENRICHMENT IN SEQUESTRATION STUDY
Predicted enrichment (ppm)
0
100
200
300
400
500
600
700
nor-adrenalin blood 200 ml
VLDL FFA
nor-adrenalin blood 200 ml
VLDL FFA
maintain at 500 ppm excess for 2 weeks
pool size = 30 l
water intake = 6 l/day
k = 0.2 /day
30 ppm excess on day 28
background = 150 ppm
0
7
14
21
28
Time after ²H₂O dose (days)
SAMPLING PROTOCOL
blood samples at 0 and 14 days
spot urines daily from day 0 to 14, also days 21 and 28
samples from days 1 to 14 can be pooled before analysis
PREDICTED ²H₂O ENRICHMENT (EXCESS OVER 150 PPM BACKGROUND)
IN THE ABSENCE OF SEQUESTRATION
0
10
20
30
40
pool size = 30 l
normal water intake = 3 l/day
k = 0.1 /day
recycling of sequestered ²H will maintain the enrichment above background and should be detected around 12 - 16 weeks after dosing
4
5
6
7
8
9
10
11
12
13
14
15
16
Time after dose (weeks)
SAMPLING PROTOCOL
Spot urine samples taken every week
from week 4 to week 16

Figure 1

MEASURED ^2H_2O ENRICHMENT IN SEQUESTRATION STUDY

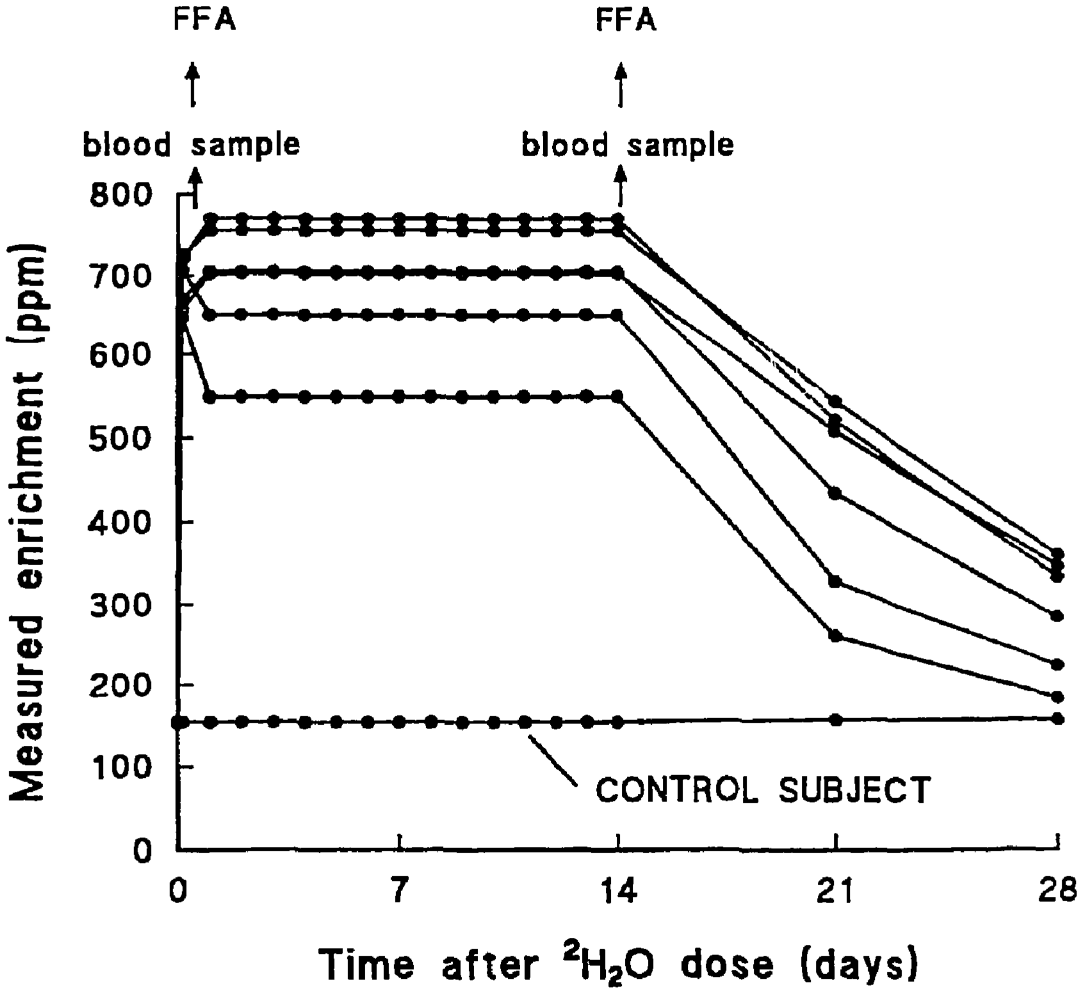

Figure 2