In Vivo Measurement of Plasma Cholesterol and Fatty Acid Synthesis With Deuterated Water: Determination of the Average Number of Deuterium Atoms Incorporated

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Fractional lipid synthesis can be measured using the incorporation of deuterium from deuterated water. The calculations require knowledge of the maximum incorporation number (N) of deuterium atoms in the molecules synthesized. For both tissue palmitate and cholesterol, N values have been found to be higher during in vivo versus in vitro experiments. We determined the N values to be used for measuring the fractional synthesis of plasma cholesterol and of palmitate triglycerides (TG). Rats were given drinking water enriched (7% to 10%) with deuterated water, and N was determined from the mass isotopomer distributions of plasma cholesterol and plasma TG palmitate and the deuterium enrichment of plasma water. We found N to be 21 for palmitate and 27 for cholesterol. These values agree with those reported for tissue palmitate and cholesterol in vivo, and are higher than values found in vitro. We also found large deuterium enrichments in plasma glucose and in liver lactate and pyruvate. We suggest that, compared with in vitro studies, in vivo metabolism of these compounds leads to an additional pathway of incorporation of deuterium into lipids through deuterium-labeled acetyl coenzyme A (CoA). This could explain why N values are higher in vivo than in vitro.

INCORPORATION into lipids of deuterium from deuterated water (^2H_2O)[1] is an attractive method for determining the fractional synthesis of fatty acids and cholesterol, especially in humans.[2-6] ^2H_2O is a safe, nonradioactive tracer, and there is no need for an intravenous infusion. Moreover, isotopic enrichment (IE) of the precursor, body water, can be easily measured,[6] especially as compared with methods using infusion of labeled acetate.[7-9] This method, like those using labeled acetate, yields the fractional synthesis, ie, the percentage of fatty acids or cholesterol in the sampled pool coming from endogenous synthesis at the time of sampling, by comparing the actual IE observed (IE obs) with the IE that would have been obtained (theoretical maximum IE) if all the molecules were derived from endogenous synthesis. This expected maximum IE (IE exp) is dependent on the IE of body water (p) and the maximum number (N) of labeled atoms that can be incorporated into the molecules synthesized (IE exp = N × p). N is 22 for hydrogen incorporation into palmitate,[10,11] but has been found to be only 14 for tritium atoms when H_2O and ^3H_2O compete for hydrogen or tritium incorporation into palmitate,[11] due to isotope effect. Deuterium is also prone to isotope effect,[12] and therefore its N value has to be determined. During in vitro studies, Lee et al[13] found N values of 17 and 20 for deuterium incorporation into palmitate and cholesterol, respectively; the value for cholesterol agrees with that reported by Javitt and Javitt.[14] However, in further in vivo studies,[15] N values of 22 for tissue palmitate and 30 for liver cholesterol were reported. This discrepancy between in vitro and in vivo studies suggested that either there is no significant isotope effect in vivo or, in addition to direct incorporation from water and through NADPH, there are other in vivo pathways contributing to deuterium incorporation into lipids.

Given the importance of using the correct N value for calculating lipid synthesis, we determined N values for plasma cholesterol and for palmitate triglycerides (TG) in rats. This was determined for plasma lipids, since plasma is the pool usually sampled in human subjects. Moreover, we searched for indications of additional in vivo pathways of deuterium incorporation into lipids. We found a large deuterium labeling of plasma glucose and of liver lactate and pyruvate that could contribute to deuterium incorporation into lipids through labeling of the methyl group of acetyl coenzyme A (CoA).

MATERIALS AND METHODS

Pyridine, *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) and bis(trimethylsilyl)trifluoroacetamide (BSTFA) were from Pierce (Rockford, IL). *O*-phenylenediamine was supplied by Prolabo (Paris, France), and ^2H_2O (99.8 atom % excess [APE]) was from Eurisotop (Gif-sur-Yvette, France). Other solvents or reactants were from Merck (Darmstadt, Germany) or Prolabo.

Male Sprague-Dawley rats (Iffa-Credo, L'Arbresle, France) weighing 250 to 340 g were placed into individual cages in an animal care facility with controlled temperature (22° ± 2°C) and light (lights on from 8 AM to 8 PM). After acclimation, they had 5 days of free access to drinking water enriched with ^2H_2O. They were fed ad libitum during the study with a standard diet (50% carbohydrates, 5% lipids, 25% proteins, 12% water, 4% cellulose, and 4% mineral salts). Two protocols were used, the first without a loading dose of ^2H_2O, and the second with a loading dose. During the first protocol, rats had free access to water enriched 7% with ^2H_2O. In a first series of experiments, blood samples were collected under ether anesthesia at 0, 8, 24, 32, 48, 72, 80, 96, and 104 hours to evaluate the kinetics of body water enrichment. In a second series of experiments (n = 6), blood was sampled only at 0, 80, 96, and 104 hours under ether anesthesia. Plasma samples were stored at −20°C until analysis. After the last blood sampling, livers were quickly exposed, freeze-clamped with tongs cooled in liquid nitro-

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gen, and stored at −80°C until analysis. During the second protocol, rats (n = 6) received an intraperitoneal loading dose of deuterated water (35 mL/kg 99% enriched ^2H_2O) and then had free access to drinking water enriched 10% with ^2H_2O. Blood was sampled at 0, 24, 48, 72, and 96 hours for measurement of plasma water IE and at 96 hours for measurement of plasma lipid IE.

Plasma lipids were extracted by the method of Folch et al[16] before separation of TG, fatty acids, phospholipids, and cholesterol by thin-layer chromatography. Briefly, 0.5 mL plasma was mixed with ethanol (1.5 mL), chloroform (3 mL), and butylhydroxytoluene (0.1 mL). The mixture was shaken and centrifuged (3,000 rpm at 4°C for 15 minutes). The upper and intermediate phases were removed, and the lower phase was evaporated to dryness under nitrogen. Extracted lipids were dissolved in 200 μL ethanol-chloroform (1:2, vol/vol) and spotted on thin-layer silica G plates (Merck, Darmstadt, Germany). Plates were developed with hexane-ether-acetic acid (80:20:1 vol/vol). Free cholesterol, TG, and fatty acids were visualized by fluorescein vapor against standards and scraped off the silica plate. Free cholesterol was eluted twice from the silica with ether, and the ether was washed with water before evaporation to dryness. The trimethylsilyl derivative of cholesterol was then prepared using pyridine (50 μL) and BSTFA (50 μL). Fatty acids were methylated and fatty acids of TG transmethylated using the method of Morrison and Smith.[17] Samples scraped from silica plates were mixed with boron trifluoride methanol and heptane-methanol and heated at 100°C for 30 minutes. The reaction was stopped by placing the samples on ice. Then the silica was precipitated with K_2CO_3 5% (1.5 mL) and fatty acid methyl esters were extracted with iso-octane. Glucose was purified from neutralized perchloric acid extract of plasma by sequential ion-exchange chromatography before preparing the bisbutylboronate-acetate derivative as previously described.[18] The quinoxalinol-*t*-butyldimethylsilyl derivative of liver pyruvate and the bis *t*-butyldimethylsilyl derivative of liver lactate were prepared as previously described.[19]

Gas Chromatography—Mass Spectrometry Analysis

All samples were injected into a gas chromatograph (HP 5890; Hewlett Packard, Palo Alto, CA) equipped with a 25-m fused silica capillary column (OV 1701; Chrompack, Bridgewater, NJ) interfaced with a mass spectrometer (HP 5871 A; Hewlett Packard) working in the electron-impact mode. The procedures for glucose, lactate, and pyruvate have been previously published.[18,19] Operating conditions for cholesterol were as follows: injector 290°C (split mode), oven 270°C, with ions of *m/z* 367, 368, 369, 370, and 371 selectively monitored. For palmitate methyl ester, the conditions were as follows: injector 250°C (split mode), oven 210°C, with ions of *m/z* 269, 270, 271, 272, and 273 monitored. Mass spectrometer parameters were set with manual tuning to obtain for the range of ion masses selectively monitored the best sensitivity and the best resolution between consecutive masses, either for palmitate or for cholesterol. Samples of unlabeled cholesterol and palmitate were run with the biological samples (all samples in triplicate). The linearity of the response of the mass spectrometer to increasing IEs was verified by running standard curves of palmitate and cholesterol enriched with known amounts of singly or doubly ^{13}C-labeled molecules. Special care was taken to obtain comparable ion peak areas (ie, <20% difference) between standard and biological samples,[20] adjusting the volume injected or diluting the sample when necessary. Coefficients of variation were less than 4% for IEs above 1% and between 5% and 8% for IEs less than 1%.

Deuterium IE in plasma water was determined by isotope ratio mass spectrometry ([IRMS] Optima; Fisons Instruments, Middlewich, England).[21] The drinking water and enriched plasma samples were diluted (1:300 and 1:100, respectively) with reference water before reduction over zinc reagent (Indiana University Foundation, Bloomington, IN) at 500°C for 30 minutes. Basal plasma samples were distilled for 4 minutes before reduction. The ^2H gas produced was analyzed on a triple-inlet IRMS unit. The $^2H/^1H$ ratio of each sample was measured six times against an international Standard Mean Ocean Water (SMOW) reference. The results were expressed as δ‰ ^2H and calculated as δ‰ = 1,000 × ([$^2H/^1H$ sample − $^2H/^1H$ reference]/$^2H/^1H$ reference), and were converted to atom percent ([AP] $^2H/[^2H + ^1H]$) for each sample, taking into account the dilution when necessary. IE in drinking water and plasma samples was expressed as APE: APE = AP sample − AP basal (AP basal is the value for plasma water before tracer administration).

Calculations

Mass isotopomer distributions of labeled palmitate and cholesterol were calculated from the observed spectral intensities of the ions monitored using matrix correction.[13] The correction matrix was obtained by running samples of unlabeled (natural) palmitate and cholesterol. For each sample, this matrix correction gives the abundance of palmitate or cholesterol molecules having incorporated one, two, three, i, excess deuterium, expressed relative to the abundance of molecules with no excess deuterium (ie, results are in molar ratio: m_i/m_o). These values, which were transformed next to molar excess, ie, the ratio of molecules having incorporated N deuterium over the total number of molecules ($m_i/[m_0 + m_1 + \ldots m_n]$), with $\Sigma m_i = 1$. From this mass isotopomer distribution, one calculates next the mean number of deuterium (IE obs, called ME by Lee et al) atoms incorporated per molecule: IE obs = $m_1 + 2\,m_2 + 3\,m_3 + \ldots nm_n$.

The comparison of IE obs with IE exp, the theoretical number of deuterium atoms that would be incorporated per molecule if the only source of molecules was endogenous synthesis, yields the contribution (F), as percentage, of synthesis to the pool of molecules sampled. IE exp is given by IE exp = p · N. p is measured by IRMS. N can be determined from the relationship between two successive isotopomers of the molecules synthesized, dependent on N and p. For a palmitate or cholesterol molecule incorporating during its synthesis N deuterium from water with an enrichment of p (p and q are the respective fractions of deuterium and hydrogen in water, with p + q = 1), the isotopomer distribution is given by the binomial distribution $(p + q)^N$. The probability of having an isotopomer, m_i, is given by

$$m_i = \frac{N!}{(N - i)!i!} \times p^i \times q^{N-i}.$$

Therefore, the ratio between two successive isotopomers is

$$\frac{m_{i+1}}{m_i} = \frac{(N - i)}{i + 1} \times \frac{p}{q},$$

which yields for m_1 and m_2,

$$m_2/m_1 = \frac{(N - 1)}{2} \times \frac{p}{q},$$

for m_3 and m_2

$$m_3/m_2 = \frac{(N - 2)}{3} \times \frac{p}{q}$$

and for m_4 and m_3

$$\frac{(N - 3)}{4} \times \frac{p}{q}.$$

These ratios are independent of any dilution of the label of synthesized compounds by unlabeled molecules from other sources. Therefore, N can be calculated from the deuterium IE of plasma water and the isotopomer distribution of the molecules synthesized.[13,15] All results are presented as the mean ± SEM.

RESULTS

First Protocol

We found in preliminary experiments that in the absence of a loading dose, stable enrichment in deuterium of plasma water was obtained from 80 to 104 hours (Fig 1). Therefore, in the next experiments we used the samples obtained at 96 hours (8 AM of day 5) to measure deuterium IE and mass isotopomer distribution in palmitate of plasma TG and in plasma cholesterol, as well as deuterium IE in plasma water, glucose, and palmitate of free fatty acids (FFA).

Mean deuterium IE in plasma water during the 80- to 96-hour period was 3.21% ± 0.1%, representing approximately 45% of the IE of drinking water. This dilution has been observed in previous studies[15,22] and has been ascribed to the entry of unlabeled water from respiratory exchange, moisture in the food consumed, and oxidation of food. Table 1 shows the mass isotopomer distribution of palmitate in plasma TG and of plasma cholesterol, the corresponding total deuterium IE (IE obs), m_2/m_1, m_3/m_2, and m_4/m_3 ratios, and the calculated values of N and F. For both palmitate and cholesterol, there was good agreement between the N values calculated using either the m_2/m_1, m_3/m_2, or m_4/m_3 ratio. The mean N value was 22 for palmitate and 27 for cholesterol. The fractions, F, of newly synthesized molecules were 0.41 and 0.44, respectively.

Second Protocol

After a loading dose, stable IE of deuterium in plasma water was obtained throughout the study (Fig 1). This IE was between 5.1% and 5.4%, thus representing about half of the IE in drinking water. Table 2 shows the isotopomer distributions of TG palmitate and plasma cholesterol, corresponding total IEs, consecutive isotopomer ratios, and calculated values of N and F. For cholesterol, neither N nor F values were different from values obtained during the first protocol. N values obtained for TG palmitate were slightly lower (20.13 to 20.68 *v* 21.84 to 22.56, $P < .10$). F values were lower (~0.23 *v* 0.41, $P < .01$). However, rats studied in the second protocol had blood sampled every day, instead of only at the end of the experiment, as in the first protocol; they consumed less food and gained less weight (~1 g/d *v* >5).

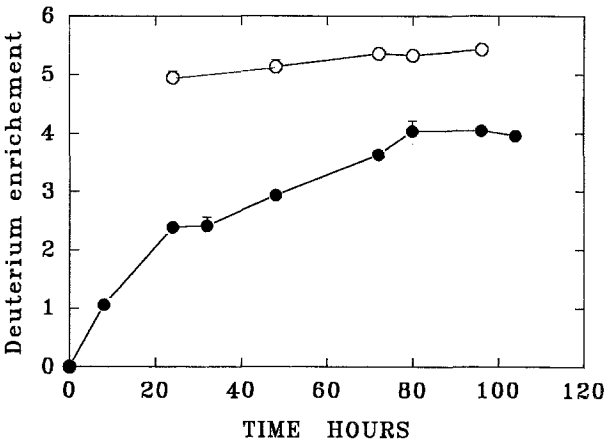

Fig 1. Evolution of deuterium IE of plasma water (expressed as $^2H_2O/[^2H_2O + H_2O]$) in rats with free access to drinking water enriched with deuterated water, without (●) and with (○) a loading dose.

Deuterium IE in Other Metabolites

Deuterium was also incorporated into other compounds. We found at the end of protocol 1 a large IE in plasma glucose and in liver lactate and pyruvate (Table 3). More surprisingly, we also found excess deuterium in palmitate of FFA. This IE was roughly two thirds of that in TG palmitate. Deuterium IE in FFA palmitate was also observed at the end of protocol 2 and was about half of that in TG palmitate. However, given the low concentration of plasma FFA, no reliable mass isotopomer distribution analysis (MIDA) could be performed for FFA palmitate.

DISCUSSION

For this study, we used MIDA of plasma cholesterol and TG palmitate to calculate the mean N of deuterium during in vivo synthesis of these molecules. Theoretically, this calculation requires a constancy of precursor IE.[23] Although plasma for analysis of cholesterol and TG palmitate labeling was collected at the end of the study, during a period of stable precursor IE, there were variations of plasma water IE throughout the study in the first protocol. This could have thereby resulted in some error in the determination of N values. Therefore, a second protocol with a loading dose of deuterated water to obtain a rapid equilibrium in plasma water was performed. N values obtained were comparable for cholesterol and were only slightly lower for TG palmitate. Therefore, the MIDA method appears relatively robust regarding the requirement for constancy of precursor IE.

Table 1. Corrected Mass Isotopomer Distribution, Total Deuterium Labeling, and Calculated N and F Values for Plasma TG Palmitate and Cholesterol (first protocol)

	m_0	m_1	m_2	m_3	m_4	$\Sigma m_i n_i$ (IE obs)	m_2/m_1	N	F	m_3/m_2	N	F	m_4/m_3	N	F
Palmitate	0.7894	0.1446	0.0510	0.0116	0.0019	0.2915	0.3461	21.84	0.412	0.2253	22.56	0.405	0.1598	22.37	0.406
	± 0.0280	± 0.0175	± 0.0079	± 0.0017	± 0.0007	± 0.0425	± 0.0220	± 0.91	± 0.048	± 0.0068	± 0.78	± 0.056	± 0.0091	± 0.89	± 0.081
Cholesterol	0.7518	0.1573	0.0671	0.0197	0.0043	0.3785	0.4244	26.58	0.447	0.2864	27.85	0.423	0.2077	28.06	0.420
	± 0.0695	± 0.0171	± 0.0085	± 0.0032	± 0.0007	± 0.0454	± 0.0213	± 0.80	± 0.041	± 0.0182	± 1.14	± 0.034	± 0.019	± 1.1	± 0.052

NOTE. Results are the mean ± SEM. N and F were calculated from the m_2/m_1, m_3/m_2, and m_4/m_3 ratios.

Table 2. Corrected Mass Isotopomer Distribution, Total Deuterium Labeling and Calculated N and F Values for Plasma TG Palmitate and Cholesterol (second protocol)

	m_0	m_1	m_2	m_3	m_4	$\Sigma m_i n_i$ (IE obs)	m_2/m_1	N	F	m_3/m_2	N	F	m_4/m_3	N	F
Palmitate	0.8417	0.0881	0.0487	0.0178	0.0042	0.2532	0.5514	20.20	0.230	0.3471	20.13	0.232	0.2540	20.68	0.228
	± 0.0179	± 0.0101	± 0.0056	± 0.0018	± 0.0004	± 0.0313	± 0.0112	± 0.18	± 0.028	± 0.0056	± 0.13	± 0.029	± 0.0093	± 0.49	± 0.032
Cholesterol	0.6697	0.1446	0.1084	0.0511	0.0189	0.5957	0.7105	25.75	0.427	0.4723	26.67	0.414	0.3601	28.11	0.394
	± 0.0161	± 0.0036	± 0.0056	± 0.0057	± 0.0007	± 0.0300	± 0.0169	± 0.49	± 0.023	± 0.0161	± 0.74	± 0.028	± 0.0159	± 1.17	± 0.026

NOTE. Results are the mean ± SEM.

The in vivo N values we found for incorporation of deuterium during synthesis of palmitate and cholesterol later exported in plasma are consistent with those reported for tissue lipids by Lee et al.[15] The F values observed for cholesterol and for TG palmitate in the first protocol after 4 days of deuterated-water administration are also consistent with those calculated by these investigators. The lower lipogenic rate during the second protocol appears to be related to the decreased food intake and body weight gain induced by repetitive blood sampling.

Our results confirm that in vivo N values for both palmitate and cholesterol are higher than those reported in vitro (17 for palmitate and 20 for cholesterol).[13-15] This suggests that compared with in vitro, there are either minor isotope effects, an unlikely hypothesis, or additional pathways contributing to deuterium incorporation in vivo. Deuterium can be incorporated either directly from water or through NADPH in the reduction of carbon-carbon double bonds. Incorporation can also occur through labeling of acetyl CoA, since 1 hydrogen atom of the odd-numbered carbon and the hydrogens of the methyl group originate from acetyl CoA.[15] Therefore, the higher in vivo N values could be explained by in vivo incorporation of deuterium in molecules whose metabolism leads to labeled acetyl CoA. One obvious candidate is glucose, since deuterium is readily incorporated during gluconeogenesis[24] and labeling on carbon 6 will lead, through glycolysis, to labeled acetyl CoA. We found large deuterium IEs in both plasma glucose and hepatic lactate and pyruvate. We did not measure deuterium IE of the lipogenic cytosolic pool of acetyl CoA. However, it is highly probable that it was labeled and contributed to deuterium incorporation into synthesized palmitate and cholesterol. In addition, between-tissue differences in the contribution of this additional pathway could explain the different N values found in liver and extrahepatic tissues by Lee et al.[15] They also suggested that the longer duration of their in vivo (1 to 8 weeks) versus in vitro (72 hours) studies played a role in this redistribution of deuterium labeling. However, our study lasted only 96 hours. Moreover, deuterium is incorporated into glucose in only a few hours.[24] Therefore, we believe that the metabolism in extrahepatic tissues plays the main role in this redistribution of labeling.

Table 3. Deuterium IE (expressed as total number of deuterium atoms incorporated per molecule, ie, $\Sigma m_i n_i$) of Plasma Glucose and Palmitate of FFA and of Liver Lactate and Pyruvate

	Glucose	Palmitate	Lactate	Pyruvate
Deuterium IE (%)	0.098 ± 0.016	0.207 ± 0.02	0.111 ± 0.014	0.110 ± 0.015

NOTE. Results are the mean ± SEM.

Use of the correct N values is important for in vivo studies of lipid synthesis, since calculation of the fractional synthesis is directly dependent on N. A N value of 14 instead of 21 for palmitate results in a decreased theoretical maximum deuterium IE and thus an increased fraction of newly synthesized palmitate. This could explain, in part, why the first estimates of lipogenesis with deuterated water[2-3] were higher than those reported using labeled-acetate infusion.[7,9] In addition, it was assumed in these studies that the only source of labeled palmitate in TG was synthesis in the liver. We found that after 4 days of deuterated-water administration, palmitate in FFA was also labeled. Since FFA taken up by liver are used for TG synthesis and secretion into very-low-density lipoproteins,[25] this recycling of label will result in overestimation of the fractional synthesis if an appropriate model is not used. However, whether significant labeling of plasma FFA also occurs in humans ingesting deuterated water remains to be established.

In conclusion, our results confirm that the in vivo N values to be used for estimation of palmitate and cholesterol synthesis are higher than the in vitro values. This difference seems related to an additional in vivo labeling through labeled acetyl CoA. In addition, during studies of liver lipogenesis, the possible recycling of label into TG through plasma FFA uptake should be considered.

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REFERENCES

1. Schoenheimer R, Rittenberg D: Deuterium as an indicator in the study of intermediary metabolism. VI. Synthesis and destruction of fatty acids in the organism. J Biol Chem 114:381-396, 1937

2. Leitch CA, Jones PJH: Measurement of human lipogenesis using deuterium incorporation. J Lipid Res 34:157-163, 1993

3. Leitch CA, Jones PJH: Measurement of triglycerides synthesis in humans using deuterium oxide and isotope ratio mass spectrometry. Biol Mass Spectrom 20:392-396, 1991

4. Jones PJH, Schoeller DA: Evidence for diurnal periodicity in human cholesterol synthesis. J Lipid Res 31:667-673, 1990

5. Jones PJH, Scanu AM, Schoeller DA: Plasma cholesterol synthesis using deuterated water in humans: Effect of short term food restriction. J Lab Clin Med 111:627-633, 1988

6. Faix D, Neese R, Kletke C, et al: Quantification of menstrual and diurnal periodicities in rates of cholesterol and fat synthesis in humans. J Lipid Res 34:2063-2075, 1993

7. Hellerstein MK, Christiansen M, Kaempfer S, et al: Measurement of de novo hepatic lipogenesis in humans using stable isotopes. J Clin Invest 87:1841-1852, 1991

8. Neese R, Faix D, Kletke C, et al: Measurement of endogenous synthesis of plasma cholesterol in rats and humans using mass isotopomer distribution analysis (MIDA). Am J Physiol 264:E134-E147, 1993

9. Hellerstein MK, Kletke C, Kaempfer S, et al: Use of mass isotopomer distributions in secreted lipids to sample lipogenic acetyl CoA pool in vivo in humans. Am J Physiol 261:E479-E486, 1991

10. Wadke M, Brunengraber H, Lowenstein JM, et al: Fatty acid synthesis by the liver perfused with deuterated and tritiated water. Biochemistry 12:2619-2624, 1973

11. Jungas RL: Fatty acid synthesis in adipose tissue incubated in tritiated water. Biochemistry 7:3708-3717, 1968

12. Lakshmanan MR, Veech RL: Measurement of rate of rat liver sterol synthesis in vivo using tritiated water. J Biol Chem 252:4667-4673, 1977

13. Lee WNP, Bassilian S, Guo Z, et al: Measurement of fractional lipid synthesis using deuterated water and mass isotopomer analysis. Am J Physiol 266:E372-E383, 1994

14. Javitt NB, Javitt JI: Cholesterol and bile acid synthesis: Utilization of D_2O for metabolic studies. Biomed Environ Mass Spectrom 18:624-628, 1989

15. Lee WNP, Bassilian S, Ajie HO, et al: In vivo measurement of fatty acids and cholesterol synthesis using D_2O and mass isotopomer analysis. Am J Physiol 266:E669-E708, 1994

16. Folch J, Lees M, Sloane-Stanley GH: A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497-509, 1957

17. Morrison WR, Smith LM: Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride methanol. J Lipid Res 5:600-608, 1964

18. Beylot M, Khalfallah Y, Riou JP, et al: Effects of ketone bodies on basal and insulin stimulated glucose utilization in vivo. J Clin Endocrinol Metab 63:9-15, 1986

19. Large V, Soloviev MV, Brunengraber H, et al: Lactate and pyruvate isotopic enrichments in plasma and tissues of post-absorptive and starved rats. Am J Physiol 268:E880-E888, 1995

20. Patterson BW, Wolfe RR: Concentration dependence of methyl palmitate isotope ratios by electron impact ionization gas chromatography–mass spectrometry. Biol Mass Spectrom 22:481-486, 1993

21. Wong WW, Lee LS, Klein PD: Deuterium and oxygen-18 measurements on microliter samples of urine, plasma, saliva and human milk. Am J Clin Nutr 45:905-913, 1987

22. Lifson NW, Little WS, Levitt DG, et al: D_2O_{18} method for CO_2 output in small mammals and economic feasibility in man. J Appl Physiol 39:657-664, 1975

23. Lee WNP, Byerley LO, Bassilian S, et al: Isotopomer study of lipogenesis in human hepatoma cells in culture: Contribution of carbon and hydrogen atoms from glucose. Anal Biochem 226:100-112, 1995

24. Landau BR, Wahren J, Chandramouli V, et al: Use of ^2H_2O for estimating rates of gluconeogenesis. J Clin Invest 95:172-178, 1995

25. Lewis GF, Uffelman KD, Szeto LW, et al: Interaction between free fatty acids and insulin in the acute control of very low density lipoproteins production in humans. J Clin Invest 95:158-166, 1995