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On problems of calculating energy expenditure and substrate utilization from respiratory exchange data

Probleme bei der Berechnung des Energieumsatzes und der Substratverwertung aus Gaswechseldaten

Summary Indirect calorimetry based on respiratory exchange measurement has been successfully used from the beginning of the century to obtain an estimate of heat production (energy expenditure) in human subjects and animals. The errors inherent to this classical technique can stem from various sources: 1) model of calculation and assumptions, 2) calorimetric factors used, 3) technical factors and 4) human factors. The physiological and biochemical factors influencing the interpretation of calorimetric data include a change in the size of the bicarbonate and urea pools and the accumulation or loss (via breath, urine or sweat) of intermediary metabolites (gluconeogenesis, ketogenesis).

More recently, respiratory gas exchange data have been used to estimate substrate utilization rates in various physiological and metabolic situations (fasting, postprandial state, etc.). It should be recalled that indirect calorimetry provides an index of overall substrate *disappearance* rates. This is incorrectly assumed to be equivalent to substrate "oxidation" rates. Unfortunately, there is no adequate golden standard to validate whole body substrate "oxidation" rates, and this contrasts to the "validation" of heat production by *indirect* calorimetry, through use of *direct* calorimetry under strict thermal equilibrium conditions.

Tracer techniques using stable (or radioactive) isotopes, represent an independent way of assessing substrate utilization rates. When carbohydrate metabolism is measured with both techniques, indirect calorimetry generally provides consistent glucose "oxidation" rates as compared to isotopic tracers, but only when certain metabolic processes (such as gluconeogenesis and lipogenesis) are minimal or / and when the respiratory quotients are not at the extreme of the physiological range. However, it is believed that the tracer techniques underestimate true glucose "oxidation" rates due to the failure to account for glycogenolysis in the tissue storing glucose, since this escapes the systemic circulation.

A major advantage of isotopic techniques is that they are able to estimate (given certain assumptions) various metabolic processes (such as gluconeogenesis) in a non-invasive way. Furthermore when, in addition to the 3 macronutrients, a fourth substrate is administered (such as ethanol), isotopic quantification of substrate "oxidation" allows one to eliminate the inherent assumptions made by indirect calorimetry.

In conclusion, isotopic tracers techniques and indirect calorimetry should be considered as complementary techniques, in particular since the tracer techniques require the measurement of carbon dioxide production obtained by indirect calorimetry. However, it should be kept in mind that the assessment of substrate oxidation by indirect calorimetry may involve large errors in particular over a short period of time. By indirect calorimetry, energy expenditure (heat production) is calculated with substantially less error than substrate oxidation rates.

Zusammenfassung Die indirekte Kalorimetrie, die auf Gaswechselmessungen beruht, ist seit Beginn des Jahrhunderts erfolgreich für die Bestimmung der Wärmeproduktion (Energieumsatz) bei Menschen und Tieren eingesetzt worden. Fehler, die mit dieser klassischen Technik verbunden sind, können von verschiedenen Quellen herrühren: 1) Modell der Berechnung und der Annahmen, 2) verwendete kalorimetri-

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sche Faktoren, 3) technische Faktoren, 4) menschliche Faktoren. Die physiologischen und biochemischen Faktoren, die die Interpretation der kalorimetrischen Daten beeinflussen, betreffen eine Änderung der Größe des Bicarbonat- und Harnstoffpools, die Akkumulation oder den Verlust (über den Atem, Urin oder Schweiß) von intermediären Metaboliten (Glukoneogenese, Ketogenese). Seit neuerer Zeit sind respiratorische Gaswechseldaten verwendet worden, um Subtratverwertungsraten in verschiedenen physiologischen und metabolischen Situationen (Fastenzustand, postprandialer Zustand etc.) zu bestimmen. Es sollte angemerkt werden, daß die indirekte Kalorimetrie einen Index für die 'overall substrate disappearance rates' liefert. In unkorrekter Weise wird dieser Index gleichgesetzt mit den Substrat'oxidations'-Raten. Bedauerlicherweise existiert kein geeigneter 'goldener' Standard, um die Ganzkörper-Substrat'oxidations'-Raten zu validieren. Im Gegensatz dazu kann die mittels indirekter Kalorimetrie gemessene Wärmeproduktion durch die direkte Kalorimetrie unter exakten thermischen Gleichgewichtsbedingungen validiert werden.

Tracertechniken, die stabile (oder radioaktive) Isotope verwenden, stellen einen unabhängigen Weg zur Bestimmung von Substratverwertungs-Raten dar. Wenn der Kohlenhydratstoffwechsel mit beiden Methoden gemessen wird, liefert die indirekte Kalorimetrie im allgemeinen Glukose"oxidations"-Raten, die mit den Tracerergebnissen übereinstimmen. Voraussetzung ist jedoch, daß bestimmte Stoffwechselprozesse (z. B. Gluconeogenese und Lipogenese) minimal sind oder/und die respiratorischen Quotienten nicht am äußersten Ende des physiologischen Bereichs liegen. Es wird jedoch angenommen, daß die Tracertechniken die wahren Glukoseoxidationsraten unterschätzen, weil die Glykogenolyse des Gewebeglukosespeichers nicht berücksichtigt wird.

Ein wesentlicher Vorteil der Isotopentracer-Techniken ist, daß sie (mit bestimmten Annahmen) verschiedene Stoffwechselprozesse (z.B. die Gluconeogenese) auf nichtinvasive Weise quantifizieren können. Schlußfolgernd kann gesagt werden, daß die Isotopentracer-Techniken und die indirekte Kalorimetrie als komplementäre Techniken betrachtet werden sollten. Es sollte beachtet werden, daß die Bestimmung der Substratoxidation mit Hilfe der indirekten Kalorimetrie große Fehler beinhalten kann, insbesondere, wenn ein kurzer Zeitraum betrachtet wird. Der Energieumsatz (Wärmeproduktion) wird mittels der indirekten Kalorimetrie mit einem wesentlich kleineren Fehler bestimmt als die Substratoxidations-Raten.

Key words Indirect calorimetry - respiratory gas exchange - energy expenditure - substrate utilization - isotope techniques

Schlüsselwörter indirekte Kalorimetrie - respiratorischer Gaswechsel - Energieumsatz - Substratverwertung - Isotopentechnik

Introduction

Indirect calorimetry based on respiratory exchange measurement has been successfully utilized from the early century to obtain an estimate of heat production (energy expenditure) in human subjects and animals. More recently, respiratory gas exchange measurements have been used to estimate substrate utilization rates in various physiological and metabolic situations (fasting, postprandial state, etc.) (26).

Tracer techniques using stable (or radioactive) isotopes, constitute an independent way of assessing substrate utilization rates. In the present report, the limitations of indirect calorimetry will be briefly reviewed and compared to the use of isotopic tracer techniques. Finally, an illustration of the application of stable isotopes in conjunction with indirect calorimetry for assessing an important metabolic process (i.e., gluconeogenesis) will be outlined.

Estimation of substrate disappearance rates by indirect calorimetry

Renewed interest in indirect calorimetry can be attributed to the fact that this technique permits not only to assess energy expenditure (13) but also the calculation of the rate of substrate utilization from the 3 primary variables (oxygen consumption VO_2, carbon dioxide production VCO_2 and urinary nitrogen excretion N_u). This calculation assumes that all substrates of endogenous and exogenous origin are completely oxidized to CO_2 and H_2O and that the main nitrogenous end product is urea. It should be mentioned that this type of calculation is not new and dates back to the fifties. In earlier metabolic investigations by British pioneers (such as Prof. R. Passmore) it has been denominated "metabolic mixture".

It should be recalled that indirect calorimetry provides an index of overall substrate *disappearance* rates but it does not provide any information upon the intermediate metabolic processes. This is incorrectly assumed to be equivalent to substrate "oxidation" rates. Unfortunately,

there is no adequate golden standard to validate whole body substrate "oxidation" rates, and this contrasts to the possible "validation" of *indirect* calorimetry, which can be made under controlled thermal equilibrium conditions by means of *direct* calorimetry.

The potential errors inherent to the classical indirect calorimetry technique can stem from various sources: model of calculation, calorimetric factors used, technical factors, and human factors. The first two factors have little influence upon the energy expenditure calculation but make a substantial impact on the calculation of substrate oxidation (25). The last two factors will obviously depend upon the technical specifications of the calorimeter (accuracy and precision) and the friability of the investigator.

Whereas the assessment of energy expenditure by indirect calorimetry is generally considered of undoubtful validity over a prolonged period of measurement, the estimation of substrate utilization is subjected to a much greater error due to the fact that it depends upon both the error of VO_2 and VCO_2 as well as the ratio VCO_2/VO_2 (i.e. the respiration quotient RQ). For example, a slight overestimation of VCO_2 will not substantially affect the calculation of energy expenditure whereas the resulting increase in RQ will lead to a large overestimation of carbohydrate "oxidation" and a concomitant underestimation of fat "oxidation". A practical example showing the dramatic effect as well as the sensitivity of substrate oxidation calculation to a minute error in VCO_2 (respectively VO_2) is given in Fig. 1. Note that the rate of urinary nitrogen excretion only provides a crude estimate of protein "oxidation" (considered as a constant in Fig. 1) but the extent to which it affects the ratio between carbohydrate and fat "oxidation" is not very large when the RQ is in the middle range, i.e., close to the RQ of protein (0.81).

The physiological and biochemical factors confounding the interpretation of calorimetric data include a change in the size of the bicarbonate and urea pools and the accumulation or loss (via breath, urine or sweat) of intermediary metabolites (18). The use of indirect calorimetry to estimate substrate utilization rate assumes that it will reflect the processes occurring at the tissue and organ levels and that no temporary storage of metabolites derived from the primary substrates occurs during the measurement. In the situation of metabolic equilibrium, when the system remains in steady state, this is expected to occur. However, under certain conditions, the calculation of fuel utilization is largely influenced by unsteady state conditions. For example, there may be a change in the size of the urea pool during an experiment. In this situation, the urinary nitrogen excretion measurement will not constitute an accurate indicator of protein "oxidation" and a correction for the change in the urea pool during the experiment is required. Practical examples of the impact of this correction for changes in both urea and bicarbonate pools have been previously outlined (26).

1) **Equation for calculating EE (26) :**

$$EE\ (kJ/min) = 15.91\ (l/min) \times VO_2 + 5.21\ (l/min) \times VCO_2$$

2) **Equation for calculating substrate oxidation*(26):**

$$\text{CHO oxidation}\ (g/min) = 4.57 \times VCO_2 - 3.23 \times VO_2 - 0.59 \times \text{prot.}$$

$$\text{lipid oxidation}\ (g/min) = 1.69 \times VO_2 - 1.69 \times VCO_2 - 0.28 \times \text{prot.}$$

Example:

Subject at rest with VO_2 = 0.25 l/min, VCO_2 = 0.2 l/min (RQ=0.8).

Variables	"true " value	overestimation of VCO_2 by 5%	overestimation of VO_2 by 5%
EE kJ/min	5.02	5.07 (+1%)	5.22 (+4%)
kcal/min	1.20	1.21	1.25
CHO oxidation g/min	0.076	0.122 (+59%)	0.036 (-47%)
Fat oxidation g/min	0.071	0.054(-24%)	0.092 (+30%)
Ratio CHO/ fat oxidation	**1.09**	**2.28**	**0.39**

*protein oxidation taken as 0.05 g/min

Fig. 1. Effect of a small systematic error (+5%) in the measurement of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) on the bias in energy expenditure (EE) and substrate (carbohydrate, CHO & fat) oxidations calculations.

One of the difficulties involved when indirect calorimetry is used is that its validity to assess substrate disappearance rate cannot be easily checked. It should be pointed out that there is no easy way to assess the accuracy and precision of substrate oxidation obtained by indirect calorimetry *in vivo*, except by comparing the values obtained with an independent technique i.e., isotopic techniques, which also have their own limitation (see further).

Influence of various metabolic processes on the calculation of substrate disappearance rate

Although under many circumstances, the disappearance of substrates from their respective metabolic pool is largely achieved by oxidation, there are also a number of situations in which a substrate is converted into another compound prior to its oxidation or a substrate is utilized for other metabolic purposes. Three examples can be briefly discussed: 1) lipogenesis from carbohydrates, 2) gluconeogenesis from aminoacids, and 3) ketogenesis from incompletely oxidized fatty acids.

In regard to 1, the process of lipogenesis from carbohydrates has a high RQ since during this process more than twice as many moles of CO_2 are produced than moles of O_2 consumed. Although in subjects eating a diet at a level close to energy equilibrium, net lipogenesis can be neglected, there are nevertheless circumstances during which this process is largely stimulated: when patients are administered a hyperosmolar glucose solution at a rate much greater than their potential glucose utilization or during carbohydrate overfeeding when the glycogen stores have been fully saturated. In this situation the rate of glucose converted into fat by "de novo" fat synthesis is greater than the rate of fat oxidation as evidenced by a non-protein RQ greater than 1.0.

The rate of carbohydrate disappearance rate assessed by indirect calorimetry represents the sum of the "true" CHO oxidation rate + that converted into fat, indicating that indirect calorimetry overestimates the actual rate of CHO oxidation when lipogenesis occurs from glucose. Note that fat concomitantly oxidized during lipogenesis is assumed to represent a homogenous mixture so that when the RQ is greater than 1.0, it is hypothesized that the same pattern of fatty acids has been synthesized as that which are contributing to oxidation (6), an assumption which is not necessarily valid.

In regard to 2, another situation in which the RQ of a given process is beyond the usual "physiological" range of 0.7 to 1.0 is the process of gluconeogenesis, i.e., the biosynthesis of glucose from glucogenic aminoacids. The average RQ of this process is very low, i.e., approximately 0.4. (24). The extent to which gluconeogenesis will affect the RQ will depend upon the source of glycogenic aminoacids and whether the neo-formed glucose is stored rather than further oxidized. The disappearance rate of CHO assessed by indirect calorimetry will represent the difference between the "true" rate of CHO oxidation minus the rate of glucose synthesis from aminoacids indicating that indirect calorimetry underestimates CHO oxidation when gluconeogenesis occurs. In contrast, the "true" rate of protein oxidation will be overestimated from the assessment of urinary nitrogen excretion since the rate of glucose synthesis by gluconeogenesis will be computed as amino-acid "oxidation"(27).

In regard to 3, the process of ketogenesis constitutes another example in which indirect calorimetry may generate erroneous values of substrate "oxidation" rates. The RQ of ketone body synthesis is zero since this process requires oxygen consumption but does not release any CO_2. The precursors of ketone bodies are fatty acids so that if the ketones are subsequently oxidized to CO_2 and H_2O, the RQ of the entire reaction will be that of fatty acid oxidation, i.e., approximately 0.7. In contrast, if the ketone bodies are transiently retained, excreted in the urine or eliminated in the breath (acetone) this process will contribute to drop the RQ to below 0.7 (24).

Estimation of substrate oxidation using tracer techniques

Isotopic labeling of substrates provides an independent way of assessing substrate utilization in man (14,20,23) and substrate turnover (29). The rate of glucose oxidation can be calculated from the $^{13}CO_2$ in expired air and the enrichment of exogenous ^{13}CHO ingested. A tracer amount of U-^{13}C glucose, in which all carbon atoms are uniformly labeled with ^{13}C, can be mixed with unlabeled glucose and given peroral as a single dose. In the simplest model, it is assumed that both the labeled and unlabeled glucose enter a single homogenous plasma glucose pool. From this pool, the glucose can either be oxidized to CO_2 and H_2O, stored as glycogen in the liver and muscles or converted to fat by lipogenesis. It should be pointed out that when the tracer is labeled with carbon, the carbon atoms are likely to be reincorporated and recycled into newly synthesized substrates.

One common assumption is that the rate of ^{13}C glucose oxidation is the same as that of unlabeled glucose. Another assumption is that all the glucose carbons are contributing equally in the oxidation of carbohydrate. However, during the oxidation of glucose, there is a differential contribution of each glucose carbon to CO_2 production: for example if glucose C-3 and C-4 contribute 100% to CO_2 elimination (in the PDH reaction), glucose C-1 and C-6 will contribute much less, at least 50% of CO_2 production (6).

One critical issue is indeed the magnitude of the label recovered in breath CO_2 which rarely approaches 100%. On the basis of ^{13}C (or ^{14}C) bicarbonate infusion tests, a test which has some inherent shortcoming (7), $^{13}CO_2$ recovery factors have ranged from 60 to 96%. This justifies the use of a correction factor (2,10,11), although on some occasions, no correction factor is applied (6). The large inter-study differences in the recovery of label in expired CO_2 can be explained by the nature of substrate labeled, the position of labeling on the molecule (31), the duration of the study as well technical factors such as the potential difficulties in the adequate preparation of the bicarbonate infusion solution in isotonic saline (6).

As opposed to the utilization of a single oral dose, a tracer dose together with a tracee can be continuously administered at a constant rate, so that a (pseudo)steady state will be achieved in the plasma ^{13}C glucose and in the $^{13}CO_2$ in expired air assuming that the glucose pool remains constant. In this situation, the rate of glucose "appearance" and "disappearance" are identical. The rate of glucose oxidation can be calculated from the fraction of the administered label recovered in expired air and the amount of glucose entering the glucose pool.

The administration of a ^{13}C labeled glucose provides an estimation of the rate of oxidation of glucose derived from the systemic circulation. Note that all sources of

glucose appearing in the blood from endogenous sources, i.e., from hepatic glycogenolysis and *de novo* glucose synthesis from aminoacids and glycerol, will be quantified by the tracer technique. This forms the basis of a new method aiming at quantifying fractional hepatic gluconeogenesis (see further). A general simplified scheme of the fate of energetic substrates in the body and the flux of substrates of interest for quantification are given in Fig. 2.

Comparison between the quantification of substrate oxidation by indirect calorimetry versus isotopic tracers

Comparison of the rate of glucose oxidation assessed by tracer isotopes has been made by a number of investigators (1,4,19,28). These have shown that glucose oxidation obtained from ^{13}C labeled glucose was lower than net carbohydrate oxidation assessed by indirect calorimetry. Two factors could explain this discrepancy: 1) The failure to account for glycogenolysis in the tissue storing glucose (i.e., muscles), since this escapes the systemic circulation and is not measured with the glucose tracer method. Given the fact that muscles accounts for approximately 20% of the resting energy expenditure and primarily oxidized non-esterified fatty acid at rest, this may constitute only part of the explanation for the discrepancy between these two methods. 2) A second factor which may play a role is, as described above, the incomplete recovery of labeled CO_2 generated from the oxidation of carbon labeled glucose due to a temporary sequestration of labeled CO_2 in body pools of slow turnover

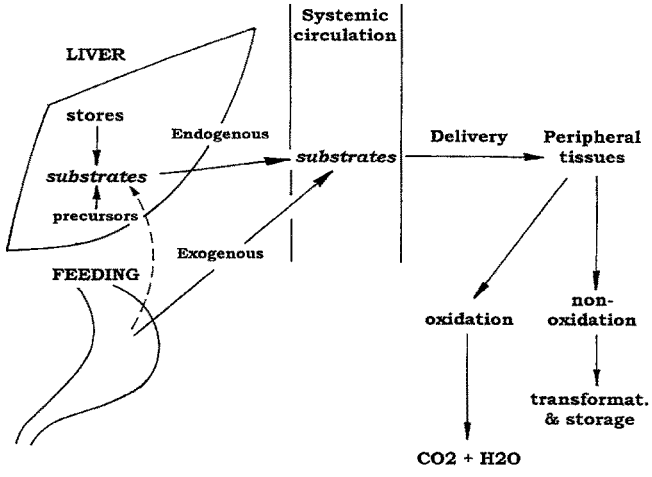

Fig. 2 Simplified diagram of the fate of energetic substrates (i.e., glucose) in the body. The flux of endogenous production and exogenous utilization can be non-invasively estimated by tracer techniques using substrates labeled with stable isotopes (^{13}C, $^{2}H_2$) in combination with indirect calorimetry, which is only capable of assessing net substrate oxidation irrespective of the metabolic pathways used.

such as bone, as well as fixation of labeled carbon in intermediary metabolism of fat, carbohydrate, and protein. 3) Other contributors to the discrepancy between the two methods include the effect of recycled labeling of glucose carbon (16, 30).

Studies carried out by Kalhan's group in pregnant women and infants have demonstrated that the discrepancy between the isotopic tracer versus the calorimetric techniques tends to widen at the upper end of the RQ's range (6) in particular during period of accelerated anabolism (growth & pregnancy). A more recent investigation has shown that estimates of carbohydrate oxidation by indirect calorimetry yielded similar values as tracer isotopes only in a narrow range of RQ's, i.e., between 0.75 and 0.90. Below and above this range of RQ's, there was a greater discrepancy between the two techniques. At high RQ, the indirect calorimetry overestimated "true" carbohydrate oxidation whereas at low RQ this was the reverse, namely indirect calorimetry underestimated "true" carbohydrate oxidation. For example, after a few days of starvation, net glucose oxidation assessed from indirect calorimetry is essentially zero (4) although the brain continues to utilize as small amount of glucose. Fig. 3 illustrates two nutritional situations leading to an underestimation (respectively overestimation) of carbohydrate oxidation.

Finally it is important to note that one can take advantage of *naturally* labeled carbohydrates with high abundance of ^{13}C atoms (such as in cane sugar or corn) which can be used, at a very low financial cost (17), for the assessment of the fate of carbohydrates ingested, in particular the partition of total CHO oxidation between its exogenous versus endogenous origin (22) . The endogenous CHO oxidation can be computed by difference between the net CHO utilization assessed by indirect calorimetry and the exogenous CHO oxidation assessed by isotopic tracer. The latter is calculated from 1) the ^{13}C enrichment (atom % excess) above basal level in the sample of $^{13}CO_2$ in expired air, 2) VCO_2 during the collection period obtained by indirect calorimetry, and 3) the natural abundance of the ^{13}C-CHO ingested.

Assessment of metabolic processes by tracer technique

As indicated above, some metabolic processes (such as gluconeogenesis) cannot be quantitatively assessed by indirect calorimetry. The use of isotopic techniques allows one to obviate this shortcoming. The assessment of lipogenesis in man by isotopomer analysis has been described by Hellerstein et al. (8). Gluconeogenesis constitutes also an important process since it appears to be the major degradative pathway for amino acids (12).

The assessment of gluconeogenesis in man has been based on labeled acetate and the determination of the enrichment (or specific activity) of phosphoenolpyruvate, the immediate precursor pool of glucose (15). However,

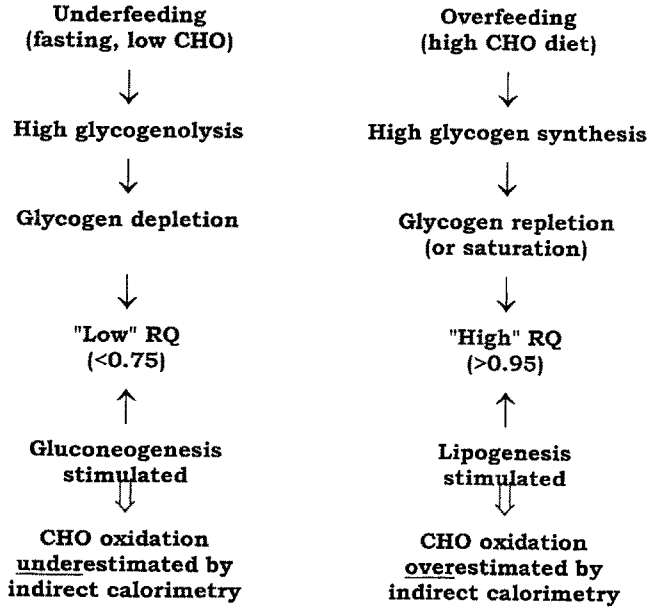

Fig. 3 Schematic diagram showing that depending upon the nutritional status (fasting vs overfeeding) and the respiratory quotient (RQ), the rate of carbohydrate (CHO) "oxidation" estimated by indirect calorimetry can either be underestimated (fasting) or overestimated (overfeeding).

this technique has been recently challenged (3) and isotopic quantification of gluconeogenesis is difficult due to the partial loss of label through various exchanges in the intermediary metabolism (9).

In our laboratory, Gay et al. have developed a new approach to assess hepatic gluconeogenesis in man in postabsorptive conditions using a non-invasive procedure (5): hepatic glycogen kinetic and hepatic gluconeogenesis were assessed by feeding subjects, for period of 5 consecutive days naturally labeled carbohydrate containing high ^{13}C abundances and by determining the ^{13}C enrichment in breath CO_2 and in plasma glucose. The principle is that the difference in isotopic enrichment between the glycogen of hepatic origin (derived from breath $^{13}CO_2$) versus the enrichment of plasma glucose is assumed to represent the isotopic dilution by endogenous glucose derived from unlabeled neoglucogenic precursors. In the postabsorptive state, the circulating plasma glucose originates from both the hydrolysis of ^{13}C glycogen and a conversion of unlabelled glycerol and amino acids into glucose.

The isotopic enrichment of glycogen from the liver can be computed as

$$^{13}C \text{ hepatic glycogen} = \frac{\text{breath } ^{13}CO_2 \cdot VCO_2}{VCO_2 \text{ (CHO)}}$$

where VCO_2 (CHO) is the carbon dioxide production obtained from the oxidation of endogenous carbohydrates.

The fractional hepatic gluconeogenesis can be calculated from the ratio between the ^{13}C plasma glucose enrichment and the ^{13}C hepatic glycogen enrichment:

$$\text{fractional gluconeogenesis} = 1 - \frac{^{13}C \text{ plasma glucose}}{^{13}C \text{ hepatic glycogen}}$$

The interpretation of this equation is that the lower the ^{13}C plasma glucose enrichment as compared to the ^{13}C hepatic glycogen enrichment, the higher the fractional gluconeogenesis. Note that this technique also permits to estimate the glycogen half-life.

When the ^{13}C naturally enriched diet was fed, resting post-absorptive breath $^{13}CO_2$ increased rapidly and reached, after 3 to 4 days, a similar enrichment value as exogenous naturally labelled carbohydrate (5). The time required to renew 50% of hepatic glycogen was estimated to be 19 ± 4 hours. Plasma ^{13}C glucose enrichment increased also rapidly and reached an apparent plateau within 2 to 3 days. However, as shown schematically in Fig. 4, plasma ^{13}C glucose enrichment at (pseudo)plateau represented about half (49± 6%) of the enrichment of glycogen oxidized at rest, which is assumed to be essentially of hepatic origin. This gave a fractional gluconeogenesis averaging 51±5% in post-absorptive conditions.

In this approach, the assumption is made that the enrichment of glycogen oxidized at rest in post-absorptive condition is essentially equal to the enrichment of hepatic glycogen. Obviously, one cannot exclude the possibility that glucose recycling providing lactate from muscle glycogen could contribute to labeling of liver glycogen. In addition, it is assumed that the rate of increase in $^{13}CO_2$ is only due to glycogen turnover. Since the ^{13}C abundance of plasma protein and VLDL remained constant

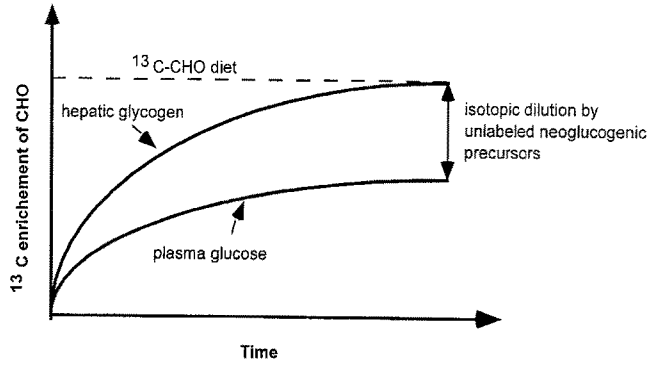

Fig. 4 Smoothed kinetic of ^{13}C enrichment of hepatic glycogen oxidized (estimated from breath $^{13}CO_2$ enrichment and indirect calorimetry) and ^{13}C plasma glucose enrichment under resting & postabsorptive conditions in young lean women. At the beginning of the study, a naturally labeled ^{13}C-carbohydrate (CHO) diet was fed for 5 consecutive days. The dotted line represents the level of ^{13}C-CHO enrichment in the diet. Note that the hepatic glycogen stores reach, after 3-4 days, the same isotopic enrichment as the exogenous ^{13}C-CHO, whereas this is not the case for the plasma ^{13}C glucose.

throughout the study, this indicates that the exogenous naturally enriched carbohydrates did not label the endogenous pools of protein and lipid.

With the present method it is unfortunately not possible to distinguish between changes in the glycogen content of liver versus skeletal muscles. In addition, part of the gluconeogenesis could be due to the Cori cycle and glucose-alanine cycle; the latter would have the same enrichment as plasma glucose due to the isotopic equilibration of labeled glucose (formed from glycogen) with lactate and alanine.

The magnitude of gluconeogenesis estimated in this study was substantially higher than that reported previously in the literature (20 - 30%). However, it was consistent with the gluconeogenesis values estimated by ^{13}C nuclear magnetic resonance in fasting conditions (21). The large methodological variability observed with this method of glycogen kinetic assessment (the coefficient of variation of fractional gluconeogenesis was 33%!) could be substantially reduced by using artificially enriched ^{13}C glucose, but at a dramatic increase in financial cost.

Conclusion

Indirect calorimetry allows one to calculate overall substrate disappearance rate by the body but does not permit to assess the flux of various intermediate metabolic processes. These processes are susceptible to influence the RQ (and hence the relative rate of fuel utilization) only when the intermediate substrates accumulate within the body or are excreted via the urine, breath or by other routes. Indirect calorimetry assesses *net* substrate utilization without taking into account the metabolic transformations, for example, gluconeogenesis from aminoacids will be measured as protein oxidation, i.e., the original substrate precursors.

The utilization of tracer isotopic technique constitutes a great advancement for the estimation of metabolic fluxes and for the quantitative assessment (given certain assumptions) of certain processes (gluconeogenesis & lipogenesis) in a non-invasive way. Furthermore, combination of indirect calorimetry and tracer techniques allows a metabolic partitioning, in the postprandial phase, of substrate oxidation according to their exogenous vs. endogenous origin. In essence, isotopic techniques and indirect calorimetry can be considered as "the two sides of the same coin": the combined utilization of indirect calorimetry and stable isotopic tracer technique will open wide areas of metabolic investigations in humans and new perspectives for assessing the turnover of substrates as well as the magnitude and importance of certain metabolic processes in various pathological and non-pathological states.

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