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REVIEW

Fasting substrate oxidation at rest assessed by indirect calorimetry: is prior dietary macronutrient level and composition a confounder?

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Indirect calorimetry, the measurement of O_2 consumption and CO_2 production, constitutes an invaluable tool as the most common method for analyzing whole-body energy expenditure, and also provides an index of the nature of macronutrient substrate oxidation—namely, carbohydrate (CHO) versus fat oxidation. The latter constitutes a key etiological factor in obesity as this condition can only develop when total fat oxidation is chronically lower than total exogenous fat intake. The standardization of indirect calorimetry measurements is essential for accurately tracking the relative proportion of energy expenditure derived from CHO and fat oxidation. Here we analyze literature data to show that the average fasting respiratory quotient typically shifts from approximately 0.80 to 0.90 (indicating a doubling of resting CHO oxidation) in response to a switch in dietary CHO intake (as % energy) from 30 to 60%. This underscores the importance of taking into account dietary macronutrient composition prior to indirect calorimetry studies in the interpretation of data on substrate utilization and oxidation.

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INTRODUCTION

The accurate and precise measurement of energy expenditure and substrate oxidation under resting conditions is at the epicenter of human metabolic research. Indirect calorimetry (VO_2 and VCO_2 measurement) has been shown to provide a reliable, noninvasive, and easy-to-perform measurement of energy expenditure, while at the same time providing an index of substrate utilization. However, the latter requires strict control of experimental conditions in order to obtain interpretable and meaningful respiration quotient ($RQ = VCO_2/VO_2$) values and hence substrate oxidation rates.

From a historical perspective, the fundamental work demonstrating the relationship between dietary composition and fasting RQ was conducted in the second half of the 1980s and early 1990s. For example, Black[1], using a room calorimeter, demonstrated that fasting RQ in well-nourished individuals in weight equilibrium is essentially identical to the RQ of the diet (called the Food Quotient (FQ)). This finding of near-equality between RQ and FQ has facilitated the application of the doubly labeled water technique for assessing total energy expenditure in free-living conditions, as this stable isotopic method tracks only CO_2 production and hence the RQ cannot be calculated in the absence of O_2 consumption measurement. As a result, the FQ value (as a proxy of RQ) can be substituted into the calculation of energy expenditure with an error not exceeding $\pm 2\%$.

Recently, the need to standardize dietary intake prior to resting energy expenditure assessment by indirect calorimetry was questioned.[2] On the basis of a review of six experimental studies published between 1994 and 2011, the authors have concluded that 'strict controls of dietary intake prior to fasting indirect calorimetry measurements may be an unnecessary burden for study participants'. Incidentally, this proposition for simplification adheres well to the general 'philosophy' of current human nutritional research, which is to streamline methodological procedures as much as possible. Because of the importance of such a conclusion for human metabolic studies, we present here an analysis of literature data pertaining to the potential impact of prior diet composition on fasting RQ at rest.

METHODS

PubMed and Google Scholar searches were conducted in October 2014 using the following Boolean phrase and key words: (indirect calorimetry) AND (substrate oxidation OR respiratory quotient OR respiratory exchange ratio) AND (diet OR macronutrient). Additional filters were used to recognize studies conducted in humans. Furthermore, the reference lists of the relevant papers were also examined to identify any further pertinent studies. The search of the literature revealed 25 experimental studies presenting data relating dietary composition to post-absorptive substrate oxidation measured by indirect calorimetry.[3–27] Given the nature of these pertinent studies (that is, different starting dietary compositions, test diet) and lack of available raw data, a traditional meta-analysis was not possible.

RESULTS

We found that, among the 25 studies relating dietary composition to post-absorptive RQ (substrate oxidation), 16 (about two-thirds) reported statistically significant changes in post-absorptive RQ, and hence substrate oxidation, with changing dietary composition. Detailed information about each study is given in the Supplementary information, along with the statistically relevant analyses. We have separated the studies into two distinct

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nutritional situations—isocaloric versus hyper-/hypo-caloric feeding— as the metabolic status of the subject influences the RQ.

To obtain a bird's eye view, graphics of the pooled studies that fed diets differing in CHO are shown in Figure 1; Figure 1a includes experimental studies in which the subjects were fed under eucaloric conditions only. Regardless of statistical significance, a clear upward trend in the relationship between the CHO content of the diet (*x* axis) and post-absorptive RQ (*y* axis), a proxy of substrate utilization, can be observed in all but two studies.[14,19] In addition, amplification of the effect of CHO intake on RQ (respectively CHO oxidation) can be seen in the studies in which a state of positive energy balance was obtained by acute overfeeding. The effects of acute supplementation or deficit in dietary CHO on fasting RQ are shown in Figure 1b.

A closer examination of individual studies (rather than pooled data for each study), where each participant constituted his/her own control, is of more interest to track interindividual variability rather than interstudy variability. This can be also visualized in Figure 2a: in this study,[16] on feeding a baseline mixed diet (45% CHO) to five lean men, an acute increase in the proportion of CHO to 72% of energy intake (keeping total energy and protein intake constant) was seen to lead to a progressive rise in post-absorptive resting RQ. A new steady state in RQ was reached within a week or so (data not shown).

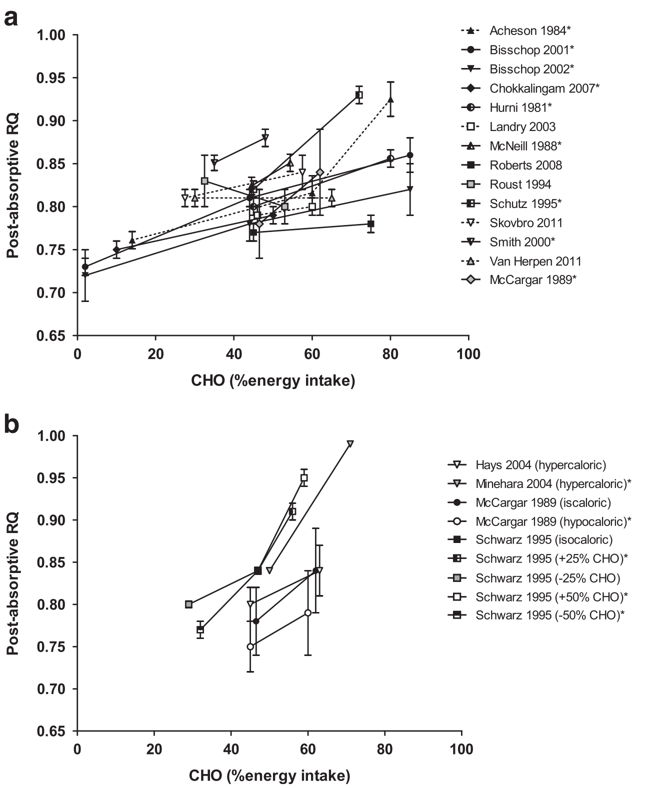

Figure 1. (**a**) Relationship between exogenous carbohydrates (dietary CHO expressed as % of energy intake) and post-absorptive respiratory quotient (RQ) in 15 experimental studies with a wide range of CHO intakes: studies under isocaloric conditions. Solid lines indicate crossover studies (that is, same subjects receiving all dietary interventions); dotted lines represent non-crossover, parallel studies (that is, different individuals in each dietary group). Data are presented as mean ± s.e. *Indicates statistically significant difference between two or more of the experimental diets. The remainder of isocaloric studies[7,11,15,20] could not be included as RQ data was not presented in the original publication. (**b**) relationship between exogenous carbohydrates (dietary CHO expressed as % of energy intake) and post-absorptive respiratory quotient (RQ) in four experimental studies with a wide range of CHO intakes: studies under acute hyper- or hypo-caloric conditions. Data are presented as mean ± s.e. *Indicates statistically significant difference from isocaloric diet. Studies involving concomitant alterations in % dietary protein[21–23] are not presented, but are summarised in the Supplementary Information.

Other results obtained in an experimental dietary intervention study on 11 healthy women of varying BMI[12] have also shown that a change in diet composition (CHO versus fat) progressively engenders a shift in RQ (hence in substrate oxidation) in order to match the higher RQ of the diet (that is, the higher FQ). These results, which are expressed as the relationship between the percentage of energy derived from CHO and post-absorptive RQ across two dietary phases (44.5% CHO+40.7% fat versus 54.4% CHO+30.8% fat), showed the same trend as the data above, although the slope response was slightly lower than that obtained in the former (Figure 2b).

DISCUSSION

The numerous factors, both exogenous and endogenous, influencing the RQ are well known and are outlined in Table 1. As far as the exogenous factors are concerned, the ratio of CHO to fat intake of the diet is the principal determinant.

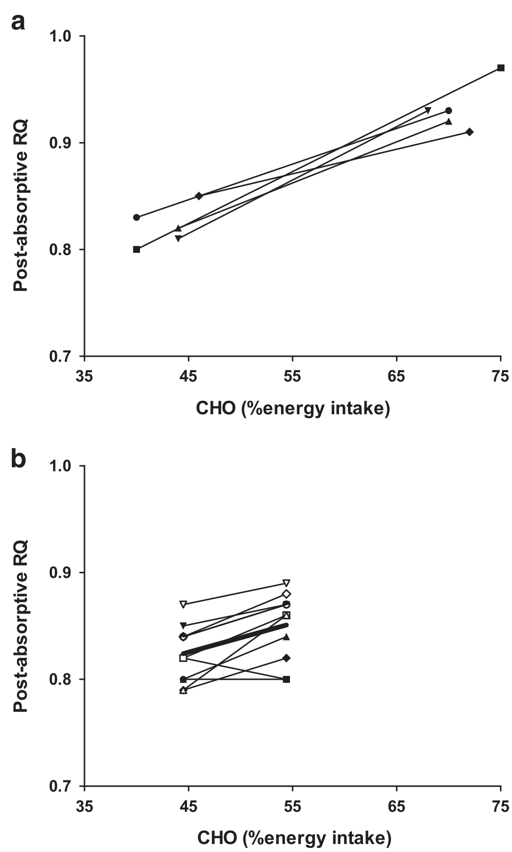

Figure 2. (**a**) Relationship between exogenous carbohydrates (dietary CHO expressed as % of total energy intake) and post-absorptive respiratory quotient (RQ) in five men fed two levels of CHO: inter-individual differences of response under isocaloric conditions (data from Schutz[16]). Lower panel (**b**): Relationship between exogenous carbohydrates (dietary CHO expressed as % of total energy intake) and post-absorptive respiratory quotient (RQ) in 11 women: inter-individual differences in isocaloric conditions (data from McNeill *et al.*[12]). The average slope response is indicated by the bold line. Note that the trend in slope response is very similar as in Fig 2a. For details regarding inter-individual variability in study participants, refer to the original article.[12]

Table 1. Multiple exogenous and endogenous factors influence the RQ in acute or chronic conditions. In acute condition, the most powerful factors are the proportion and the total amount of carbohydrate intake, and the proportion of CHO to fat intake (that is, the FQ)

	Effect on resting (RQ)		*Example reference*
Exogenous			
Dietary composition (CHO vs fat)	↑	↓	As reviewed (Supplementary Table)
Endogenous			
Increased glycogen stores	↑		28
Decreased glycogen stores		↓	35
Positive energy balance (excess energy)	↑		36
Negative energy balance (energy deficit)		↓	36
Increased total fat mass (obesity)	↓		37
Physiological states			
Growth (anabolic)	↓		38
Pregnancy (third trimester)	↔	↑	39
Metabolic processes			
Gluconeogenesis		↓	40
De novo lipogenesis	↑		41
Physical activity			
Training (chronic)		↓	42
Genetic factors/race			43
Physiopathological			
Type 1 diabetes		↓	44

Overall, in this global analysis, we found a positive relationship between %CHO in the diet and post-absorptive RQ (and hence macronutrient oxidation) under both normo- and under/overfeeding conditions.

In the latter situation, there was an upward shift in the slope of this relationship depending on excess energy intake over energy requirement. This steeper slope is explained by the surfeit absolute CHO intake, increasing glycogen storage, enhancing insulin secretion and stimulating *de novo* lipogenesis. Note that the flexibility of RQ is enormous during extreme CHO overfeeding, increasing post-absorptive values from 0.77 while on a low CHO diet/high fat diet (to deplete glycogen stores) to an *RQ* much above 1.0 (approximately 1.15), a 'non-physiological' value explained by the process of net *de novo* lipogenesis.[28] Indeed, the conversion of part of the exogenous CHO into fat (which generates a theoretical RQ of 2.75) is very powerful for increasing the overall 'physiological' RQ.

Although the majority of literature data demonstrate a clear upward trend in the relationship between dietary CHO content and post-absorptive RQ, not all studies reported a significant relationship between the two. It is possible that several of these 'no-effect' studies may be explained as being false negative. Indeed, failure to find a statistically significant effect of macronutrient intake on RQ may be due to several factors:

a) large inter-individual variability resulting from a parallel study design, with the control group not comprising the same subjects as the 'active' group[10,22]; b) an insufficient duration (<30 min continuous measurement) of indirect calorimetry measurement—that is to say, failure to reach a physiological steady state or a ventilatory disequilibrium such as an induction of hyperventilation state[14]; c) a dietary intervention not being long enough to stabilize the RQ[7,13]; d) the magnitude of change (delta) in dietary macronutrient ratio not large enough for substrate oxidation to adjust sufficiently to attain a steady state and/or to pick up the small anticipated effect on substrate oxidation.[7,10,15,21] Finally, it is important to note that in two studies that failed to show an upward trend in the relationship between % dietary CHO and postprandial RQ, key methodological information was not provided, such as the duration of resting energy expenditure measurements and length of fasting.[14,19] Furthermore, the study by Van Herpen *et al.*[19] estimated energy and nutrient intake retrospectively rather than fully controlling it *per se*, and Roust *et al.*[14] utilized a mouthpiece and nose clip rather than a ventilated hood system, with measurements being taken in the evening. Caution should be taken when interpreting studies utilizing a mouthpiece and nose-clip system, as this apparatus has been shown to elicit hyperventilation[29–33] and increased CO_2 production[34], and hence may elevate RQ. Although Roust *et al.*[14] used a practice session to acclimatize the participant, and those who were 'unable to relax during the measurements, as manifested by unusually high post-absorptive minute ventilation rates and respiratory quotients' were excluded, the order of the diets was fixed and as such a learning effect cannot be ruled out.

PERSPECTIVES AND CONCLUSIONS

In conclusion, evidence to date indicates that the nature of dietary macronutrient composition does affect post-absorptive substrate oxidation in subsequent resting conditions. In contrast, there is no evidence that slight variations in macronutrient food composition, prior to measurement by indirect calorimetry, influence the post-absorptive resting energy expenditure under isocaloric conditions. Taken together, results pertaining to the magnitude of RQ in the post-absorptive state at rest may well be providing us with valuable information concerning a subject's qualitative dietary intake and metabolic status, with a high CHO/low fat utilization (high RQ) suggesting a greater proportion of CHO to fat in the diet (high FQ). However, one of the major difficulties in RQ measurement and interpretation is technical: accurately determining RQ by indirect calorimetry is not an easy task as this quotient is very sensitive to slight errors in the numerator (VCO_2) and in the denominator (VO_2). Even worse, these errors may occur in opposite directions, further amplifying the error in the resultant RQ and FQ calculation. As precise RQ measurement is essential to accurately calculate the rate of substrate oxidation, the proposed suggestion to abandon the control of food intake prior to indirect calorimetry measurements (at least the standardization of the last meal on the previous day) is questionable. This clearly emphasizes the need for researchers to obtain complete and accurate information regarding participants' diets prior to investigations of whole-body substrate utilization. Alternatively, another approach (often used) is to feed the patients under supervision in the laboratory or under metabolic ward conditions 24 h per day.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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