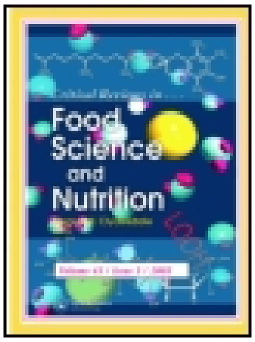

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A review of the effect of dietary composition on fasting substrate oxidation in healthy and overweight subjects

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Running title: Dietary effects on substrate utilisation

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Abstract

Aim: The purpose of this review was to assess existing evidence on the effects of chronic dietary macronutrient composition on substrate oxidation during a fasted state in healthy and overweight subjects. *Methods:* A systematic review of studies was conducted across five databases. Studies were included if they were English language studies of human adults, ≥19 years, used indirect calorimetry (ventilated hood technique), specified dietary macronutrient composition and measured substrate oxidation. *Results:* There was no evidence that variations of a typical, non-experimental diet influenced rate or ratio of substrate utilisation, however there may be an upper and lower threshold for when macronutrient composition may directly alter preferences for fuel oxidation rates during a fasted state**.** *Conclusion**:*** This review indicates that macronutrient composition of a wide range of typical, non-experimental dietary fat and carbohydrate intakes has no effect on fasting substrate oxidation. This suggests that strict control of dietary intake prior to fasting indirect calorimetry measurements may be an unnecessary burden for study participants. Further research into the effects of long-term changes in isocaloric macronutrient shift is required.

Keywords: Obesity, indirect calorimetry, substrate oxidation.

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Introduction

Indirect calorimetry is a non-invasive method for estimating energy expenditure and rate of macronutrient utilisation (Battezzati, 2001). It provides a measure of oxygen consumption and carbon dioxide production to calculate resting energy expenditure and the respiratory quotient (RQ). The RQ is calculated as the ratio of carbon dioxide expired to oxygen inspired (RQ=CO2/O2) and indicates which macronutrient substrate (fat or carbohydrate) is the predominant fuel source undergoing oxidation (Matarese 1997, Ferrannini 1988). The ratio of substrate oxidation is of interest to clinical nutrition research to investigate potential mechanisms of metabolic conditions such as obesity, diabetes and non-alcoholic fatty liver disease (NAFLD)(Ferrannini, 1988).

Indirect calorimetry is a sought-after method for metabolic studies due to the high quality of information collected and its non-invasive approach. It is a technique used increasingly in populations of patients with obesity-related disease as a tool to identify metabolic disruptions in fuel oxidation capacity, glucose disposal pathways, and potential effects of treatment options such as weight loss interventions. Weight reduction has been demonstrated to improve the metabolic profile of obesity by increasing insulin sensitivity and facilitating a greater flexibility in substrate utilisation during fasted and fed states (Corpeleijn et al., 2008). The increased demand for indirect calorimetry generates a need to validate its use in different metabolic states, and to identify potential confounding to control for in the research setting. Historically, studies

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using indirect calorimetry to determine macronutrient substrate utilisation have strictly controlled the dietary intake of participants in the days leading up to measurement due to a belief that variability in diets may influence the rate of substrate oxidation while fasting. Setting standardised diets prior to calorimetry testing imposes a significant burden and cost to the study and compliance is difficult to ascertain if not closely supervised. In 1997, Schrauwen et al.(Schrauwen et al., 1997) demonstrated in fed-state healthy participants using a whole room respiration chamber, that a significant increase in dietary fat intake can produce adaptations in fat oxidation rates within seven days. However, the preference of substrate utilisation during a fasted state was not specified. It is important to identify if there is evidence that typical dietary composition can influence preference of substrate utilisation in a fasted state so that study methodologies can ensure robust validity of calorimetry measures.

The specific research question 'does dietary composition have an effect on substrate oxidation in the fasted state in healthy or overweight individuals?' was addressed in this review. Therefore the aim is to assess the existing evidence for the effect of chronic dietary macronutrient composition on substrate oxidation rate during the fasted state.

Methods

Search strategy and selection criteria

Keywords used as the search terms for the present study included a combination of: (energy intake, diet composition) AND (substrate oxidation, indirect calorimetry). Additionally manual searches were used to retrieve relevant papers from bibliographies of the articles included in the review. The literature search was conducted using five databases (PUBMED, EMBASE,

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CINAHL, Scopus, and the Cochrane Library). The initial search was limited only by species and age of subjects (human adults ≥19 years).

Results

Figure 1 illustrates the pathway and outcomes for the search strategy which initially resulted in 36 articles. From these, five were reviews and seven did not use indirect calorimetry in the methodology and were therefore excluded. Manual searches from bibliographies resulted in the addition of six relevant papers, leaving 25 potentially relevant articles for inclusion in the review. Of these studies, five used alternative methods of measuring substrate oxidation, two included participants with chronic medical conditions, two did not specify dietary macronutrient composition, and one did not specify substrate oxidation rates and therefore were excluded from the review. The final 6 articles included in the review were graded using the National Health and Medical Research Council (NHMRC) levels of evidence grading (NH&MRC, 2009), and rated using the American Dietetic Association Evidence Analysis quality criteria checklist (ADA, 2009). All relevant articles are summarized in Table 1. Results are presented according to body mass index (BMI) classification of: (i) healthy weight; (ii) overweight or obese (See Table 1).

Healthy weight subjects

Only two randomised crossover design studies captured data from healthy weight participants. The BMI range of healthy subjects included in this review was 21-29kg/m2, and the age range was 26-55 years (Bisschop et al., 2001; Roberts et al., 2008). The diet macronutrient composition of the prescribed diets differed between studies. Table one outlines the macronutrient composition of each study. Bisschop et al. (Bisschop et al., 2001) used three study diets ranging

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from no fat (0%) to moderate (43%) and high (83%) fat content. After consumption of each diet for 11 days, it was found that the rate of fat oxidation during fasting was significantly higher ($p<0.05$) after following the diet with 83% energy from fat compared to the other diets (Bisschop et al., 2001). Roberts et al. (Roberts et al., 2008) applied two different study diets comparing 10% low fat to 40% moderately high fat. After 3 days of consuming each diet it was found that there was no significant effect of diet on RQ or substrate oxidation when fasted (Roberts et al., 2008).

Overweight/obese subjects

Of the studies that included overweight or obese subjects, the BMI ranged from 27-35 kg/m2, and age ranged from 27-80 years (Roust et al., 1994; Hays et al., 2004; Westerbacka et al., 2005; van Herpen et al., 2011). The upper age range of the overweight group was older than that of the healthy group due mostly to one study by Hays et al. (Hays et al., 2004) which included a subject group aged 55-80 years. The macronutrient composition of prescribed diets varied considerably among studies (Table 1). Hays et al. (Hays et al., 2004) compared two different study diets, a low fat (18%) and a moderate fat (41%) diet with and without additional prescription of exercise. The diets were consumed for 14 weeks and no significant change in RQ was observed between baseline and post intervention after either diet irrespective of exercise (Hays et al., 2004). Roust et al.(Roust et al., 1994) also used two study diets of typical dietary composition reflecting moderate high fat (40-45%) and reduced fat (27%). After two weeks consuming diet one, and four weeks consuming diet two, it was found that RQ was not different between groups and did not change significantly in response to diet two (Roust et al., 1994). Westerbacka et al. (Westerbacka et al., 2005) used two diets (high fat 56% and low fat 16%) and found that after 14

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days on each diet, total energy expenditure, lipid, and CHO oxidation during fasting remained unchanged. Lastly, Van Herpen et al. (van Herpen et al., 2011) compared males randomly allocated to 3 weeks intake of either 20% low fat intake or 55% high fat intake (van Herpen et al., 2011). Similar to the other studies, it was demonstrated that RQ during the fasted state was no different between groups (van Herpen et al., 2011).

Discussion

The aim of this review was to assess existing evidence on the effects of chronic dietary macronutrient composition on substrate utilisation during a fasted state in healthy and overweight subjects. Based on the NHMRC levels of evidence grading (NH&MRC, 2009), and the American Dietetic Association Evidence Analysis quality criteria checklist (ADA, 2009) several high quality studies used scientifically robust methodologies including randomised crossover studies to test the effect of dietary composition on fasting substrate utilisation in both healthy and overweight individuals.

A range of similar studies that were directed at testing the effect of macronutrient proportion in the diet on the rate of substrate oxidation achieved consistent results with the exception of one study. The literature that focused on the relationship between diet and substrate oxidation primarily indicated there was no significant effect of the diet on substrate utilisation when consuming a typical, non-experimental diet (Roust et al., 1994; Hays et al., 2004; Westerbacka et al., 2005; Roberts et al., 2008; van Herpen et al., 2011). One exception was the well designed randomised cross over study by Bisschop et al. (Bisschop et al., 2001) which observed a twofold increase in fasting fat oxidation after 11 days of a high fat (83%) diet (refer to table 1). This dietary composition, with almost an absence of carbohydrate as a fuel source (~2%), and over

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double the fat composition of the average diet of the study population (Hulshof et al., 1993) was only achieved through the use of a specially designed liquid supplement for research purposes. It does not represent a dietary fat composition realistically achieved through regular food sources which typically ranges between 25-45% energy from fat for adult populations regardless of gender (Elmadfa, 2009). Cuisine patterns and therefore dietary fat content is not static from day to day and are likely to vary between individuals under free living conditions (Tapsell et al., 2004) but this variation is likely to remain within two standard deviations of a population mean (up to 43% energy from fat) which still represents half the fat content of what was offered under the research conditions.The results indicate that within a healthy lean population, fat and carbohydrate oxidation rates are adaptable to a lack of one fuel source (Bisschop et al., 2001). This suggests that there may be an upper and lower threshold for when macronutrient composition may directly alter preferences for fuel oxidation rates during a fasted state. These results should be interpreted with caution as there were only two studies that represented a healthy lean population included in this review and therefore may not generalise to the broader population. Further research is required to confirm an upper and lower level of macronutrient composition where effects on fasting substrate oxidation may be observed. This contrasts current scientific dogma that dietary composition induces considerable variability in calorimetry measures and brings into question the need for standardised dietary prescription in the days leading up to assessment if subjects follow typical dietary patterns.

Confounding factors such as age, BMI, and gender must be considered when interpreting these studies. Fat oxidation is generally lower in the elderly (>60 years, BMI <30kg/m2) compared to younger, healthy weight subjects (<35years, BMI < 25kg/m2) (Levadoux et al., 2001). The lower fat oxidation in the elderly may be explained by age-related changes in body composition such as lower fat-free mass (FFM) and reduced energy expenditure compared to younger individuals (Levadoux et al., 2001). The age of subjects included in this review were similar across studies, with the exception of Hays et al. (Hays et al., 2004) who studied participants 55-80 years of age, but who demonstrated results consistent with other studies in younger age groups. Fat oxidation may be altered in obesity (Bugianesi et al., 2005; Perseghin et al., 2006) and therefore the effect of BMI of participants included in studies may be relevant, although there were no significant differences in fasting fat oxidation between lean and overweight subjects consuming typical macronutrient patterns. The manuscripts identified in this review focus predominantly on obese participants, with limited studies (n=2) involving lean participants (Bisschop et al., 2001). While these two studies were of high quality with robust methodologies, validation in a wider selection of lean healthy populations is warranted... Female subjects were over represented in this review and while any gender difference is likely to be due to differences in energy expenditure between males and females (Levadoux et al., 2001), there were no gender differences reported in the outcomes of these studies.. The study by Bisschop et al. (Bisschop et al., 2001) which demonstrated fat oxidation adaptations in the absence of carbohydrate intake was performed in males only. This is unlikely to be due to gender difference but rather extreme diet conditions that are most likely only achieved in a research setting. Further research is required to determine whether gender plays a role in the metabolic response to changes in dietary intake.

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The duration of dietary intervention is an important aspect of any study designed to address the question of macronutrient effects on substrate utilisation. Schrauwen et al. (Schrauwen et al., 1997) prescribed an isoenergetic diet (30% energy from fat) for 6 days, followed by an increased fat diet (60% energy from fat) for seven days, and measured substrate oxidation by respiration chamber. It was found that when in energy balance lean subjects were capable of adjusting fat oxidation to fat intake within seven days of when dietary fat content was increased. However the lower physical activity levels mandated within a respiration chamber were a possible confounder (Schrauwen et al., 1997). All of the diet interventions in this review exceeded seven days except for that of Roberts et al. (Roberts et al., 2008) in which the study diets were followed for a period of three days. While it may be argued that this could be inadequate duration to detect differences in fat oxidation, the results were consistent with those studies of similar dietary composition and longer duration.

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Conclusion

This review indicates that macronutrient composition of a wide range of typical, non-experimental dietary fat and carbohydrate intakes has no measurable effect on fasting substrate oxidation. This greatly informs future research methodologies by suggesting that strict controls of dietary intake prior to fasting indirect calorimetry measurements may be an unnecessary burden for study participants.

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Figure 1

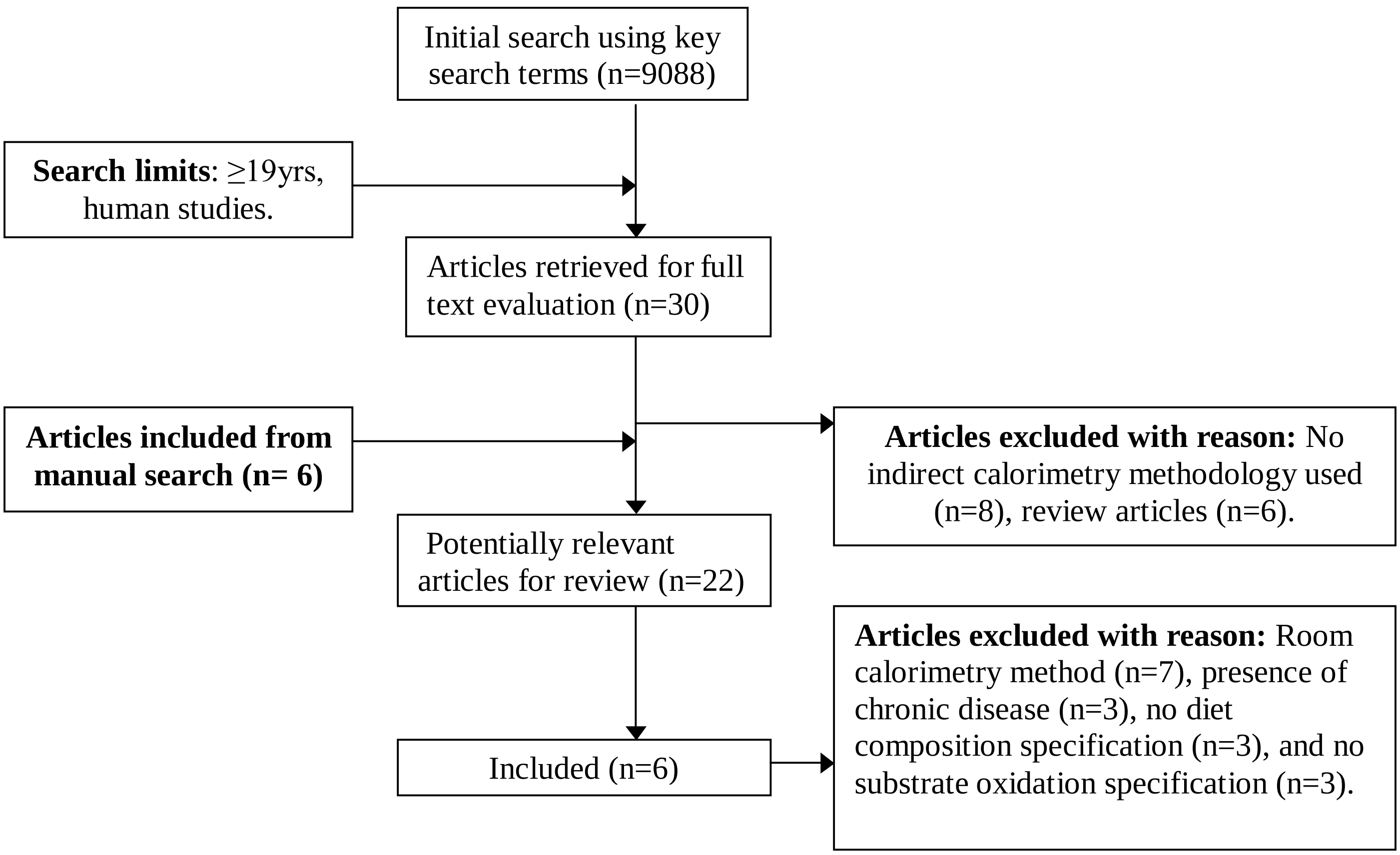

Figure Headings

Figure 1: Search methodology flowchart. Studies were included if they were English language studies of human adults, ≥ 19 years, used indirect calorimetry (ventilated hood system), specified dietary macronutrient composition and measured substrate oxidation

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Table 1 Clinical studies investigating the effect of diet on substrate oxidation.

Author (year) Country	Study design NHMRC level of evidence APA Quality Rating	No. of subjects	Study group	Intervention	Duration	Main results	Comments
HEALTHY SUBJECTS							
Bisschop et al. (2001)(Bisschop, de Metz et al. 2001) Amsterdam	Randomised crossover design II +	6	BMI range 21-26kg/m^2; age range 29-55 years; healthy males; no family history of diabetes; no use of medications.	Studied on 3 occasions after consuming study diet for 11 days, Diet 1= 0% fat + 85% CHO, Diet 2= 41% fat + 44% CHO, Diet 3= 83% fat +2% CHO (all isocaloric); Indirect calorimetry ventilated hood technique was used for VO_2 & VCO_2 measurements continuously in supine position at basal insulin levels after 14 hr fast for 30 mins and hyperinsulinemic state for 30 mins.	24 weeks (including 8-10 week washout)	Rate of fat oxidation significantly increased after diet 3 when compared to other 2 diet groups at basal insulin concentrations and at hyperinsulinemic state ($p< 0.05$).	Males only, Diet 1 & 3 improbable as realistic diet.

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Roberts et al. (2008)(Roberts, Bickerton et al. 2008) United Kingdom	Randomised crossover design II +	8	BMI mean 24 kg/m^2 (range 22-29kg/m^2); Mean age 43 years (range 26-53 years); healthy volunteers, 6 female & 2 male.	Diet A= 40% fat, 45% CHO; Diet B = 10% fat, 70% CHO (all isocaloric). Diet consumed 3 days prior to metabolic investigation; ventilated hood technique used for indirect calorimetry in fasted and postprandial periods; 20 min reading taken before meal and 20 min readings taken hourly after meal for 6 hrs.	8 weeks (including 6 week washout period)	No significant effect of diet on RQ or relative substrate oxidation when fasted.	Diet B is improbably as realistic diet.
OVERWEIGHT/OBESE SUBJECTS							
Hays et al. (2004)(Hays, Starling et al. 2004) United States of America	Randomised Control Trial II +	34 (12 control, 11 high CHO, 11 high CHO+ exercise)	Mean BMI matched across groups at 31kg/m^2; age range 55-80 years; 20 female, 14 males; diagnosed impaired glucose	Initial diet composition (wk 1) 35% fat, 45% CHO; baseline measure taken; Control diet - 41% fat, 45% CHO, High CHO diet - 18% fat, 63% CHO (all isocaloric); REE measured week 1 & 14 with ventilated hood	14 weeks	No significant change in RQ observed between baseline and post intervention diet group, or diet and exercise group.	Study diets were followed for longer period of time in comparison to other studies, older participants.

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			tolerance; no meds known to affect glucose metabolism.	technique in fasted state 30 min measurement.			
Roust et al. (1994)(Roust, Hammel et al. 1994) United States of America	Non randomised crossover experimental trial III-2 -	23 (8 non-obese control, 15 overweight/obese group)	BMI 30-35; age range 34-40 years; female; low physical activity; weight stable; no medications known to affect energy metabolism.	Diet 1 for 2 weeks (baseline diet) = 40-45% fat, 30-35% CHO; Diet 2 for 4 weeks = 27% fat, 53% CHO (all isocaloric). REE and breath CO_2 were measured at baseline for 30 minutes before test meal was administered.	6 weeks	RQ was not different between groups and did not change significantly in response to diet 2.	Females subjects only, no washout period, does not specify if fasted during baseline REE measurements.
Westerbacka et al. (2005)(Westerbacka, Lammi et al. 2005) Finland	*Randomised crossover design* II +	10	Mean BMI = 33; mean age 43± 5 years; female; elevated LFT's was not exclusion criteria, no	Participants consumed 2 different isocaloric diets for 14 days each containing16% or 56% energy from fat. Indirect calorimetry performed by hood	4 weeks	Rates of total energy expenditure, lipid, and CHO oxidation remained unchanged between diets after an overnight fast.	Females only, no washout period.

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			known acute or chronic illness; no use of meds that may alter glucose metabolis m.	technique. Hood was placed on subject 10 mins before measurement started, calculations of substrate oxidation according to Ferrannini, 1988.			
van Herpen et al. (2011)(van Herpen, Schrauwen-Hindering et al. 2011) Netherlands	Randomised Control Trial II -	20 (10 low-fat diet, 10 high-fat diet)	Mean BMI 28.5 ± 0.6 kg/m^2; mean age 55.5 ± 2.5; male; overweight but otherwise healthy subjects.	All subjects consumed low-fat diet (20% fat, 65% CHO) for first 3 weeks. Half of subjects (randomly allocated) switched to high fat diet (55% fat, 30% CHO) for remaining 3 weeks. Diets isocaloric. Insulin sensitivity measured by hyperinsulinemic euglycemic clamp at 3 weeks and six weeks. Fat and CHO oxidation calculated by Frayn method.	6 weeks	RQ was not different after low or high fat diet.	No washout period in between study diets, males only.

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BMI, Body Mass Index; CHO, Carbohydrate; VO_2, Oxygen utilisation (ml/min); VCO_2, Carbon dioxide production (ml/min); REE, Resting Energy Expenditure; RQ, Respiratory Quotient; CO_2, Carbon Dioxide; LFT, Liver Function Test; - , poor quality; +, good quality.

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