European Journal of Clinical Nutrition (2009) 63, 78–86

0954-3007/09 $32.00
www.nature.com/ejcn

ORIGINAL ARTICLE

A prospective study of dietary carbohydrate quantity and quality in relation to risk of ovulatory infertility

JE Chavarro[1,2], JW Rich-Edwards[2,3,4], BA Rosner[2,5] and WC Willett[1,2,4]

[1]*Department of Nutrition, Harvard School of Public Health, Boston, MA, USA;* [2]*Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA;* [3]*Division of Women's Health and Connors Center for Women's Health and Gender Biology, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA;* [4]*Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA and* [5]*Department of Biostatistics, Harvard School of Public Health, Boston, MA, USA*

Objective: To evaluate whether the amount or quality of carbohydrate in diet is associated with ovulatory infertility.
Subjects and Methods: In total, 18 555 married, premenopausal women without a history of infertility were followed as they attempted a pregnancy or became pregnant during an 8-year period. Diet was assessed two times during follow-up using a validated food-frequency questionnaire and prospectively related to the incidence of infertility due ovulatory disorder.
Results: During follow-up, 438 women reported ovulatory infertility. Total carbohydrate intake and dietary glycemic load were positively related to ovulatory infertility in analyses adjusted for age, body mass index, smoking, parity, physical activity, recency of contraception, total energy intake, protein intake and other dietary variables. The multivariable-adjusted risk ratio (RR) (95% confidence interval (CI)) of ovulatory infertility comparing the highest-to-lowest quintile of total carbohydrate intake was 1.91 (1.27–3.02). The corresponding RR (95% CI) for dietary glycemic load was 1.92 (1.26–2.92). Dietary glycemic index was positively related to ovulatory infertility only among nulliparous women. Intakes of fiber from different sources were unrelated to ovulatory infertility risk.
Conclusions: The amount and quality of carbohydrate in diet may be important determinants of ovulation and fertility in healthy women.
European Journal of Clinical Nutrition (2009) **63,** 78–86; doi:10.1038/sj.ejcn.1602904; published online 19 September 2007

Keywords: diet; carbohydrates; glycemic index; glycemic load; infertility; ovulation

Introduction

Infertility is a common condition affecting up to 15% of couples during their reproductive lifetime (Hull *et al*., 1985). In the United States alone seven million women have an impaired ability to bear children (Chandra *et al*., 2005). Despite the magnitude of the problem, few modifiable risk factors for infertility have been identified and therefore prevention of this condition has been largely ignored.

Much evidence suggests that insulin sensitivity may be an important determinant of ovulatory function and fertility (Hjollund *et al*., 1999; Azziz *et al*., 2001; Vrbikova *et al*., 2002). Both the quality and quantity of carbohydrate in diet influence glucose metabolism, affecting insulin demand or sensitivity in healthy individuals (Jeppesen *et al*., 1997; Murakami *et al*., 2006) as well as in diabetics and women with polycystic ovary syndrome (PCOS) (Garg *et al*., 1994; Douglas *et al*., 2006a). However, it is not currently known whether the effects of carbohydrate intake on glucose and insulin metabolism lead to changes in ovulatory function or fertility in healthy women. To answer this question we prospectively evaluated whether dietary glycemic index, glycemic load and the intakes of carbohydrates and fiber from different sources were associated with infertility due to anovulation in a group of apparently healthy women.

Correspondence: Dr JE Chavarro, Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, USA.
E-mail: jchavarr@hsph.harvard.edu
Received 26 April 2007; revised 29 July 2007; accepted 6 August 2007; published online 19 September 2007

Subjects and methods

The Nurses' Health Study II (NHS II) started in 1989 when over 116 000 female registered nurses completed a mailed questionnaire. Participants have been followed since then with mailed questionnaires every other year. Here, we present a prospective analysis of incident ovulatory infertility among participants of this cohort who provided dietary information as part of their participation in the NHS II. The study was approved by the Institutional Review Board of Brigham and Women's Hospital.

Follow-up for the current analysis started in 1991, when diet was first measured, and concluded in 1999. Every 2 years participants were asked if they had tried to become pregnant for more than 1 year without success since the previous questionnaire administration, and to indicate whether their inability to conceive was caused by tubal blockage, ovulatory disorder, endometriosis, cervical mucous factor, male factor, was not found, was not investigated or was due to another reason. In a validation substudy, self-reported diagnosis of ovulatory infertility was confirmed by review of medical records in 95% of the cases (Rich-Edwards *et al*., 1994). Women were also asked if they became pregnant during the preceding 2-year period, including pregnancies resulting in miscarriages or induced abortions. Using this information we simulated a cohort of women attempting to become pregnant. Only married women, with available dietary information and without a history of infertility, were eligible to enter the analysis. These women contributed information to the analysis during each 2-year period in which they reported a pregnancy or a failed pregnancy attempt, and were followed until they reported an infertility event from any cause, reached menopause or underwent a sterilization procedure (themselves or their partner), whichever came first. Ten diabetic women met these criteria. Since the small number of diabetics would preclude meaningful statistical adjustment or exploration of modification of the associations by diabetes, diabetic women were excluded from the analysis. After exclusions, we identified 18 555 women without a history of infertility who tried to become pregnant or became pregnant during the study period.

Women who met the selection criteria for the study and reported infertility due to ovulatory disorder were considered cases. All other events (pregnancies—resulting in live births, miscarriages or induced abortions—and infertility due to other causes) were considered non-cases.

Dietary assessment

Dietary information was collected in 1991 and 1995 using a validated food-frequency questionnaire (FFQ) with 133 and 142 food items, respectively (Willett *et al*., 1985; Liu *et al*., 2001). Participants were asked to report how often, on average, they consumed each of the foods and beverages included in the FFQ during the previous year. The questionnaire offered nine options for frequency of intake, ranging from never or less than once per month to six or more times per day. Nutrient intakes were estimated by summing the nutrient contribution of all food items in the questionnaire. The nutrient content of each food and specified portion size was obtained from a nutrient database derived from the US Department of Agriculture (2001) with supplemental information from food manufacturers and, in the case of glycemic index, from previously published databases (University of Sydney (Australia), 2002; Jenkins *et al*., 1981; Miller *et al*., 1995). Each woman's average dietary glycemic index was calculated by summing the product of the carbohydrate content of each food times its frequency of intake and glycemic index, divided by the total carbohydrate intake (Salmeron *et al*., 1997). To account for differences in overall carbohydrate intake we also calculated dietary glycemic load as the product of total carbohydrate intake times the average dietary glycemic index (Salmeron *et al*., 1997). The percentage of energy contributed by carbohydrates was calculated as the intake of energy from this nutrient divided by total energy intake. Dietary glycemic index, glycemic load and intakes of fiber and other nutrients were adjusted for total energy intake using the nutrient residual method (Willett and Stampfer, 1998).

Statistical analyses

The risk ratio (estimated as an odds ratio) of ovulatory infertility in relation to dietary factors was estimated using logistic regression. The generalized estimating equation approach (Fitzmaurice *et al*., 2004) with an exchangeable working correlation structure, was used to account for the within-person correlation in outcomes at different time periods. We divided women into five groups according to quintiles of dietary glycemic index, glycemic load and intakes of total carbohydrates and fiber. In these models, the risk ratio was computed as the risk of infertility in a specific quintile of cumulative averaged intake compared to the risk in the lowest quintile. Tests for linear trend were conducted by using the median values of intake in each category as a continuous variable. The risk ratio associated with a 1-serving per day increase in consumption of specific carbohydrate-rich foods was estimated by modeling the intake of these foods as a continuous variable. All models were adjusted for total energy intake, age and calendar time at the beginning of each questionnaire cycle. Multivariable models included additional terms for body mass index (wt (kg) ht^{-2} (m)) (BMI), parity, smoking history, physical activity, history of oral contraceptive use and dietary factors found to be related to infertility in preliminary analyses (use of multivitamins and intakes of alcohol, coffee, iron, *trans*-fatty acids, animal and vegetable protein) (Chavarro *et al*., 2006, 2007, in press). Multivariable models for total carbohydrate intake were fit with adjustment for protein and *trans*-fatty acid intakes to simulate the substitution of carbohydrates for naturally occurring fats (saturated, mono-unsaturated and poly-unsaturated), and without adjustment for protein and *trans*-fatty acid intakes to simulate the substitution for the average mixture of protein and fats in the study population. Values of the dietary and non-dietary variables were updated as new data became available from follow-up questionnaires.

Lastly, we examined whether the associations between dietary variables and ovulatory infertility were modified by participant characteristics (age, parity and BMI), or the presence of long menstrual cycles (>40 days), by introducing cross-product terms between carbohydrate and the variable of interest. All *P*-values were two sided. Analyses were performed in SAS version 9.1.

Results

Between 1991 and 1999, 26 971 eligible pregnancies and pregnancy attempts were accrued among 18 555 women. Of these, 3430 (12.7% of all events) were incident reports of infertility, of which 2165 were women reporting at least one medical diagnosis for infertility and 438 (1.6% of all events, 20.2% of investigated infertility cases) were incident reports of ovulatory infertility. Higher carbohydrate intake at baseline was associated with a generally healthy lifestyle. Women who consumed more carbohydrates, also consumed less fat, animal protein, alcohol and coffee while consuming more protein from vegetable sources, fiber and multivitamins (Table 1). These women were also less likely to be smokers, weighed less and were more physically active than women with lower carbohydrate intake. On the other hand, while women with a high glycemic index diet also consumed less saturated fat, animal protein, alcohol and coffee, they also had a higher intake of *trans*-fat, lower intakes of fiber and multivitamins and were less physically active than women with lower glycemic index diets.

Total carbohydrate intake was unrelated to ovulatory infertility in models simulating the substitution of carbohydrates for the average mixture of other energy sources in the study population (Table 2). Nevertheless, there was a positive association between total carbohydrate intake and ovulatory infertility in the models where this nutrient is increased at the expense of naturally occurring fats. In the multivariable-adjusted model, women in the highest quintile of total carbohydrate intake had a 78% greater risk of ovulatory infertility than women in the lowest quintile (RR (95% CI) = 1.78 (1.14–2.78)) and there was a statistically significant linear trend towards greater risk of ovulatory infertility with increasing carbohydrate intake (*P*, trend = 0.005). Further adjustment for cereal fiber intake made this association even stronger.

Dietary glycemic index and glycemic load were unrelated to ovulatory infertility in age and energy-adjusted analyses (Table 2). After adjustment for potential confounders, particularly after adjustment for animal and vegetable protein intake, dietary glycemic load was associated with a higher risk of infertility due to anovulation. Statistical adjustment for potential confounders had no impact in the results for dietary glycemic index. Similarly, total fiber intake and intake of fiber from different sources were unrelated to ovulatory infertility both in age and energy-adjusted analyses and in multivariable-adjusted analyses (Table 3).

We then examined the association between the main carbohydrate foods sources in this population and ovulatory infertility. Overall, high glycemic index foods (cold breakfast cereals, white rice and potatoes) were associated with a greater risk of ovulatory infertility while low glycemic index foods (brown rice, pasta and dark bread) were associated with a reduced risk of this condition (Table 4). This pattern was not consistent across all foods examined, however, and only the association between cold breakfast cereal intake and ovulatory infertility was statistically significant ($P = 0.02$).

Lastly, we examined whether the associations of dietary glycemic index, glycemic load, total carbohydrate intake and fiber intake with ovulatory infertility were different in subgroups of women defined by age, menstrual cycle length, BMI and parity. Age modified the association between fiber intake, and particularly cereal fiber intake and ovulatory infertility (*P*, interaction = 0.02). An increase in cereal fiber intake of $10\,g\,day^{-1}$, while holding caloric intake constant, was associated with a 44% lower risk of ovulatory infertility ($P = 0.02$) among women older than 32 years while the same increase in cereal fiber intake was unrelated to infertility due to anovulation among younger women ($P = 0.78$). Parity appeared to modify the associations of fiber intake and dietary glycemic index with ovulatory infertility. Fiber intake was associated with a small, yet not statistically significant, reduction in the risk of ovulatory infertility among nulliparous women while it was unrelated to this disease among parous women (Table 5). Dietary glycemic index was positively related to the risk of ovulatory infertility among nulliparous women but unrelated to this condition among parous women (*P*, interaction = 0.02). The risk ratio (95% CI) comparing top to bottom quintiles of dietary glycemic index was 1.55 (1.02–2.37) among nulliparous women (*P*, trend = 0.05) and 0.78 (0.51–1.18) among parous women (*P*, trend = 0.22).

Discussion

We prospectively examined the associations of the quality and quantity of dietary carbohydrates with ovulatory infertility among a group of apparently healthy women and found that the quantity of carbohydrate was positively related to this condition when consumed at the expense of naturally occurring fats. In addition, we found a positive association between the quality of carbohydrate and ovulatory infertility among nulliparous women. These associations were independent of other characteristics of diet previously found to be associated with fertility and insulin sensitivity (Liese *et al*., 2005; Chavarro *et al*., 2006, 2007). We have previously reported how factors known to increase insulin resistance such as overweight and obesity, physical inactivity and intake of *trans*-fatty acids (Lefevre *et al*., 2005; The Diabetes Prevention Program Research Group, 2005), are

Table 1 Age-standardized baseline[a] characteristics of the study population by quintiles of total carbohydrate and glycemic index intake

	Carbohydrate intake			*Glycemic index*		
	Q1	*Q3*	*Q5*	*Q1*	*Q3*	*Q5*
Age, years	32.5	32.5	32.9	33.2	32.5	32.0
Saturated fat intake, g day^{-1}	26.8	22.3	16.9	23.3	22.4	21.0
Monounsaturated fat intake, g day^{-1}	28.0	23.2	18.0	23.2	23.5	23.0
Polyunsaturated fat intake, g day^{-1}	12.3	10.8	9.0	10.8	10.9	10.5
Trans unsaturated fat intake, g day^{-1}	3.7	3.1	2.4	2.8	3.1	3.3
Animal protein intake, g day^{-1}	76.0	64.1	49.9	71.3	63.9	57.7
Vegetable protein intake, g day^{-1}	19.8	22.5	24.7	21.6	22.7	22.1
Fiber intake, g day^{-1}	15.4	18.1	21.1	19.0	18.3	16.6
Cereal fiber intake, g day^{-1}	4.5	5.9	7.1	5.4	5.9	5.8
Alcohol intake, g day^{-1}	4.4	2.6	1.8	4.2	2.8	1.7
Coffee intake $\geqslant$2 cups per day^{-1}, %	30	23	18	32	23	16
Multivitamin use, %	48	58	63	59	58	53
Current smoker, %	11	5	6	8	6	8
Body mass index, kg m^{-2}	24.8	23.8	23.0	24.2	23.9	23.6
Physical activity, METs per week	17.8	20.6	27.0	26.1	20.9	16.9
Cycles $\geqslant$40 days, %	3	4	4	4	3	4
Nulliparous, %	23	21	29	28	22	21
Oral contraceptive use at the beginning of the mailing cycle, %	20	16	14	16	16	17

[a]Values are presented as age-standardized means and proportions. Values for age are not age-standardized.

also associated with a greater risk of ovulatory infertility (Rich-Edwards *et al.*, 1994, 2002; Chavarro *et al.*, 2007). Others have found that greater levels of HbA1c are associated with decreased fertility and subclinical metabolic characteristics resembling PCOS among apparently healthy women (Hjollund *et al.*, 1999). Further, insulin sensitizers improve reproductive metabolic parameters and ovulatory function in PCOS women (Moghetti *et al.*, 2000; Brettenthaler *et al.*, 2004). Our results are consistent with these previous findings and with the hypothesis that insulin sensitivity may be a key factor regulating ovulatory function and fertility in healthy women.

Although we are unaware of previous studies examining the association between carbohydrate intake and ovulatory infertility in apparently healthy women, studies among women with PCOS, the most common cause of anovulation, suggest that the amount and quality of carbohydrate in diet may influence reproductive function. In a small retrospective case–control study, Douglas *et al.* (2006b) found that women with PCOS had a greater intake of high glycemic index foods, particularly white bread and fried potatoes, than age, race and BMI-matched controls. In a small feeding trial among women with PCOS conducted by the same group (Douglas *et al.*, 2006a), consuming a low carbohydrate diet (43 vs 56% of energy) led to changes that would be expected to result in improved reproductive and metabolic outcomes including significant reductions in fasting and post glucose challenge insulin levels and a reduction in free testosterone levels of borderline statistical significance (Douglas *et al.*, 2006a). Our results are consistent with the findings of these two studies.

We found that total carbohydrate intake and dietary glycemic load were positively related to ovulatory infertility and that dietary glycemic index was associated to this condition among nulliparous women. Since greater insulin sensitivity and improved glucose homeostasis have been previously linked to improved ovulatory function and fertility in healthy women and women with PCOS (Hjollund *et al.*, 1999; Azziz *et al.*, 2001; Brettenthaler *et al.*, 2004), it is possible that the observed associations are mediated through the effects of carbohydrate intake on glucose metabolism. In randomized crossover feeding trials among healthy subjects, increasing total carbohydrate intake, in an amount equivalent to going from the lowest to the highest quintile of intake in this study population, increased post-prandial insulin response to test meals after a few days on this diet (Coulston *et al.*, 1983) as well as day-long insulin and glucose concentrations after 3 weeks of following this diet (Jeppesen *et al.*, 1997). Similar effects have been observed in type 2 diabetics (Garg *et al.*, 1994; Chen *et al.*, 1995) PCOS women (Douglas *et al.*, 2006a) and hypertriglyceridemic individuals (Liu *et al.*, 1983). Low-glycemic index diets significantly decrease HbA1c levels and improve insulin sensitivity among diabetics (Brand-Miller *et al.*, 2003; Jimenez-Cruz *et al.*, 2003; Rizkalla *et al.*, 2004), women with impaired glucose tolerance (Ostman *et al.*, 2006) and are related to fasting glucose and HbA1c levels among healthy women (Murakami *et al.*, 2006). In addition, higher dietary glycemic load has been associated with higher fasting glucose levels (Murakami *et al.*, 2006) and greater insulin resistance (McKeown *et al.*, 2004), although these findings have not been consistent across studies (Lau *et al.*, 2005; Liese *et al.*, 2005; Mayer-Davis *et al.*, 2006). Increased insulin levels resulting from greater carbohydrate intake could also lead to increased free IGF-I and androgen levels as a consequence of insulin action on the production of IGF-I, its binding proteins and SHBG (Kaaks and

Table 2 Risk ratios and 95% confidence intervals for ovulatory infertility by quintiles of carbohydrate, glycemic index and glycemic load intake

	Quintile of intake					P *trend*[a]
	1	*2*	*3*	*4*	*5*	
Total carbohydrate						
Median intake (% of calories)	42	47	51	54	60	
Cases/non-cases	92/5302	90/5304	79/5316	90/5305	87/5306	
Substitution for fats and protein						
Age and energy adjusted RR[b]	1.00 (referent)	1.01 (0.76–1.36)	0.90 (0.66–1.22)	1.03 (0.77–1.38)	1.00 (0.74–1.35)	0.99
Multivariable adjusted RR 1[c]	1.00 (referent)	1.08 (0.80–1.46)	1.00 (0.73–1.37)	1.18 (0.87–1.61)	1.03 (0.74–1.41)	0.74
Substitution for naturally occurring fats						
Age and energy adjusted RR[d]	1.00 (referent)	1.16 (0.85–1.58)	1.11 (0.79–1.55)	1.37 (0.96–1.94)	1.54 (1.02–2.33)	0.03
Multivariable adjusted RR 2[e]	1.00 (referent)	1.28 (0.93–1.76)	1.30 (0.92–1.85)	1.69 (1.17–2.45)	1.78 (1.14–2.78)	0.005
Multivariable adjusted RR 3[f]	1.00 (referent)	1.30 (0.94–1.80)	1.35 (0.94–1.92)	1.78 (1.22–2.60)	1.91 (1.21–3.02)	0.003
Glycemic index						
Median intake	50	53	54	56	58	
Cases/non-cases	111/5285	79/5325	68/5329	82/5298	98/5296	
Age and energy adjusted RR[b]	1.00 (referent)	0.73 (0.54–0.97)	0.62 (0.46–0.84)	0.75 (0.56–1.00)	0.88 (0.67–1.16)	0.35
Multivariable adjusted RR 2[e]	1.00 (referent)	0.82 (0.61–1.10)	0.72 (0.52–0.99)	0.90 (0.66–1.22)	1.08 (0.79–1.48)	0.68
Multivariable adjusted RR 3[f]	1.00 (referent)	0.82 (0.61–1.10)	0.72 (0.52–0.99)	0.90 (0.66–1.23)	1.09 (0.79–1.48)	0.67
Glycemic load						
Median intake	100	114	123	133	149	
Cases/non-cases	94/5297	102/5298	69/5322	73/5320	100/5296	
Age and energy adjusted RR[b]	1.00 (referent)	1.10 (0.83–1.47)	0.74 (0.54–1.01)	0.77 (0.57–1.05)	1.04 (0.78–1.38)	0.65
Multivariable adjusted RR 2[e]	1.00 (referent)	1.44 (1.07–1.96)	1.02 (0.73–1.44)	1.25 (0.85–1.82)	1.82 (1.21–2.75)	0.02
Multivariable adjusted RR 3[f]	1.00 (referent)	1.47 (1.08–1.99)	1.05 (0.75–1.48)	1.29 (0.88–1.90)	1.92 (1.26–2.92)	0.01

[a]Calculated in a separate regression model with median protein intake in each quintile as a continuous variable and the same group of covariates specified for the corresponding model.
[b]Adjusted for age (continuous), calendar time (four 2-year intervals) and total energy intake (continuous).
[c]Age and energy adjusted model further adjusted for body mass index (<20, 20–24.9, 25–29.9, ⩾30 and missing), parity (0, 1, ⩾2 and missing), smoking history (never, past 1–4 cig day^{-1}, past 5–14 cig day^{-1}, past 15–24 cig day^{-1}, past ⩾25 cig day^{-1} or unknown amount, current 1–4 cig day^{-1}, current 5–14 cig day^{-1}, current 15–24 cig day^{-1} and current ⩾25 cig day^{-1} or unknown amount), physical activity (<3 MET-h week^{-1}, 3–8.9 MET-h week^{-1}, 9–17.9 MET-h week^{-1}, 18–26.9 MET-h week^{-1}, 27–41.9 MET-h week^{-1}, ⩾42 MET-h week^{-1} and missing), oral contraceptive use (current user, never user, past user 0–23 months ago, past user 24–47 months ago, past user 48–71 months ago, past user 72–95 months ago, past user 96–119 months ago, past user ⩾120 months ago and missing), frequency of multivitamin use (non-users, ⩽2 tablets week^{-1}, 3–5 tablets week^{-1}, ⩾6 tablets week^{-1} and missing), intake of alcohol (no intake, <2 g day^{-1}, 2–4.9 g day^{-1}, ⩾5 g day^{-1}), coffee (<1 serving per month, 1 serving per month, 2–6 servings per week, 1 serving per day, 2–3 servings per day, ⩾4 servings per day) and iron (quintiles).
[d]Age and energy adjusted model further adjusted for *trans*-fatty acids (continuous), animal protein (continuous) and vegetable protein (continuous).
[e]Multivariable adjusted model 1 further adjusted for *trans*-fatty acids (continuous), animal protein (continuous) and vegetable protein (continuous).
[f]Multivariable adjusted model 2 further adjusted for cereal fiber intake.

Lukanova, 2001), thus creating an endocrine environment similar to that suggested to be responsible for the clinical manifestations of PCOS (Dunaif, 1997; Ehrmann, 2005). Whether these or other mechanisms explain the observed associations should be explored in future studies.

An alternative explanation for our results is that the positive association between increasing carbohydrate intake at the expense of natural fats and ovulatory infertility is not a result of metabolic effects of carbohydrate intake but rather due to a beneficial effect on ovulatory function of consuming naturally occurring fats. This possibility is consistent with our previous findings of an inverse association between intakes of specific fatty acids, particularly of saturated and mono-unsaturated fats (Chavarro *et al*., 2007) and with previous reports suggesting that intakes of total and saturated fat may have beneficial effects on menstrual cycle characteristics and ovulatory function (Hill *et al*., 1984; Deuster *et al*., 1986; Snow *et al*., 1990; Reichman *et al*., 1992). Although we cannot distinguish between a beneficial effect of naturally occurring fats and an adverse effect of carbohydrates from these data, this distinction is not practically important because, with total calories fixed, an increase in one nutrient largely implies a decrease in the other.

Our study has several strengths including its prospective design. Dietary information was collected 2–4 years before outcome events were reported, minimizing the possibility that our results were affected by fertility status at the time information on diet was obtained. The use of previously validated instruments for the assessment of diet and infertility due to ovulatory problems add strength to our results. The most important limitation is that our study was

Table 3 Risk ratios and 95% confidence intervals for ovulatory infertility by quintiles of fiber intake

	Quintile of intake					P trend[a]
	1	2	3	4	5	
Total fiber						
Median intake, g day^{-1}	12	15	17	20	24	
Cases/non-cases	84/5213	91/5412	85/5359	90/5278	88/5271	
Age and energy adjusted RR[b]	1.00 (referent)	1.05 (0.78–1.42)	1.00 (0.73–1.35)	1.07 (0.79–1.45)	1.03 (0.75–1.40)	0.86
Multivariable adjusted RR 1[c]	1.00 (referent)	1.14 (0.83–1.56)	1.11 (0.78–1.56)	1.22 (0.84–1.77)	1.26 (0.81–1.95)	0.31
Multivariable adjusted RR 2[d]	1.00 (referent)	1.15 (0.84–1.57)	1.11 (0.78–1.57)	1.22 (0.84–1.78)	1.23 (0.79–1.91)	0.38
Cereal fiber						
Median intake, g day^{-1}	3	4	5	7	9	
Cases/non-cases	85/5224	109/5555	93/5108	66/5327	85/5319	
Age and energy adjusted RR[b]	1.00 (referent)	1.21 (0.91–1.61)	1.10 (0.82–1.49)	0.76 (0.55–1.05)	0.94 (0.69–1.27)	0.13
Multivariable adjusted RR 1[c]	1.00 (referent)	1.34 (0.98–1.82)	1.25 (0.90–1.74)	0.86 (0.59–1.26)	1.10 (0.74–1.63)	0.64
Multivariable adjusted RR 2[d]	1.00 (referent)	1.31 (0.96–1.79)	1.20 (0.86–1.68)	0.82 (0.56–1.20)	1.00 (0.67–1.49)	0.33
Vegetable fiber						
Median intake, g day^{-1}	3	5	6	7	10	
Cases/non-cases	89/5422	73/5311	96/5193	92/5329	88/5278	
Age and energy adjusted RR[b]	1.00 (referent)	0.84 (0.61–1.14)	1.13 (0.84–1.51)	1.07 (0.79–1.43)	1.02 (0.75–1.38)	0.56
Multivariable adjusted RR 1[c]	1.00 (referent)	0.90 (0.66–1.25)	1.21 (0.89–1.63)	1.11 (0.81–1.53)	1.04 (0.72–1.51)	0.64
Multivariable adjusted RR 2[d]	1.00 (referent)	0.92 (0.67–1.27)	1.24 (0.92–1.68)	1.15 (0.84–1.59)	1.10 (0.76–1.60)	0.44
Fruit fiber						
Median intake, g day^{-1}	1	2	3	4	6.1	
Cases/non-cases	84/5309	94/5404	87/5286	79/5210	94/5324	
Age and energy adjusted RR[b]	1.00 (referent)	1.08 (0.81–1.46)	1.05 (0.78–1.42)	0.96 (0.71–1.31)	1.09 (0.80–1.47)	0.88
Multivariable adjusted RR 1[c]	1.00 (referent)	1.18 (0.87–1.60)	1.14 (0.83–1.56)	1.08 (0.78–1.50)	1.20 (0.86–1.67)	0.49
Multivariable adjusted RR 2[d]	1.00 (referent)	1.18 (0.87–1.60)	1.14 (0.83–1.56)	1.08 (0.78–1.50)	1.19 (0.85–1.66)	0.52

[a]Calculated in a separate regression model with median protein intake in each quintile as a continuous variable and the same group of covariates specified for the corresponding model.
[b]Adjusted for age (continuous), calendar time (four 2-year intervals) and total energy intake (continuous).
[c]Age and energy adjusted model further adjusted for body mass index (<20, 20–24.9, 25–29.9, ⩾30 and missing), parity (0, 1, ⩾2 and missing), smoking history (never, past 1–4 cig day^{-1}, past 5–14 cig day^{-1}, past 15–24 cig day^{-1}, past ⩾25 cig day^{-1} or unknown amount, current 1–4 cig day^{-1}, current 5–14 cig day^{-1}, current 15–24 cig day^{-1} and current ⩾25 cig day^{-1} or unknown amount), physical activity (<3 MET-h per week, 3–8.9 MET-h per week, 9–17.9 MET-h per week, 18–26.9 MET-h per week, 27–41.9 MET-h per week, ⩾42 MET-h per week and missing), oral contraceptive use (current user, never user, past user 0–23 months ago, past user 24–47 months ago, past user 48–71 months ago, past user 72–95 months ago, past user 96–119 months ago, past user ⩾120 months ago and missing), frequency of multivitamin use (non-users, ⩽2 tablets week^{-1}, 3–5 tablets week^{-1}, ⩾6 tablets week^{-1} and missing), intake of alcohol (no intake, <2 g day^{-1}, 2–4.9 g day^{-1}, ⩾5 g day^{-1}), coffee (<1 serving per month, 1 serving per month, 2–6 servings per week, 1 serving per day, 2–3 servings per day, ⩾4 servings/day), iron (quintiles) *trans*-fatty acids (continuous), animal protein (continuous) and vegetable protein (continuous).
[d]Multivariable adjusted model 1 further adjusted glycemic load.

not a cohort of women known to be planning a pregnancy. Cases were clearly trying to conceive, but some pregnancy non-cases may have conceived accidentally. Nevertheless, the study was conducted in a cohort of women whose pregnancies are likely to be planned given their socio-economic characteristics (that is professional women in their late 20 s and early 30 s) (Chandra *et al*., 2005), and was restricted to married women, whose pregnancies are even more likely to be intentional (Chandra *et al*., 2005). We also considered women diagnosed with infertility from other causes as non-cases, making it less likely that pregnancy intention affected our results. Another potential limitation is the possibility that our findings are due to unmeasured factors related both to carbohydrate intake and ovulatory infertility. However, we accounted statistically for a variety of known and suspected risk factors for infertility in our analyses and only two of these factors (protein intake and parity) had major impact on the results.

In conclusion, we found that greater carbohydrate intake and dietary glycemic load were associated with an increased risk of infertility due to anovulation in a cohort of apparently healthy women and that dietary glycemic index was positively related to this condition among nulliparous women in this cohort. These findings are consistent with multiple lines of evidence suggesting a role of insulin and glucose metabolism on fertility but they need to be confirmed by other studies. In the meantime, as lower intakes of refined starch are associated with reduced risks of major chronic diseases (Liu *et al*., 2000; Schulze *et al*., 2004; Oh *et al*., 2005), reducing intakes of carbohydrates from these sources is sensible for women attempting to become pregnant as it may also improve fertility.

Table 4 Risk ratios (95% CIs) of ovulatory infertility associated with increasing the intake of specific carbohydrate-rich foods by 1 serving day^{-1}

Food	*Age and energy-adjusted*[a]		*Multivariable-adjusted*[b]	
	RR (95% CI)	P	*RR (95% CI)*	P
Cold breakfast cereal	1.10 (0.88–1.38)	0.39	1.31 (1.04–1.63)	0.02
White rice	1.24 (0.84–1.82)	0.28	1.19 (0.78–1.81)	0.41
Brown rice	0.83 (0.36–1.94)	0.67	0.73 (0.29–1.84)	0.50
Pasta	0.95 (0.57–1.58)	0.84	0.92 (0.52–1.62)	0.78
Potatoes (baked, boiled or mashed)	1.34 (0.82–2.18)	0.24	1.27 (0.80–2.03)	0.32
French fried potatoes	1.97 (0.87–4.49)	0.10	1.74 (0.63–4.81)	0.29
White bread	0.91 (0.77–1.07)	0.24	0.98 (0.83–1.15)	0.80
Dark bread	0.90 (0.79–1.03)	0.14	0.99 (0.86–1.15)	0.93
English muffins or bagels	1.08 (0.76–1.54)	0.67	0.97 (0.67–1.41)	0.87
Muffins or biscuits	0.80 (0.42–1.52)	0.50	0.71 (0.37–1.33)	0.28

[a]Adjusted for age (continuous), calendar time (four 2-year intervals) and total energy intake (continuous).
[b]Age and energy adjusted model further adjusted for body mass index (<20, 20–24.9, 25–29.9, ⩾30 and missing), parity (0, 1, ⩾2 and missing), smoking history (never, past 1–4 cig day^{-1}, past 5–14 cig day^{-1}, past 15–24 cig day^{-1}, past ⩾25 cig day^{-1} or unknown amount, current 1–4 cig day^{-1}, current 5–14 cig day^{-1}, current 15–24 cig day^{-1} and current ⩾25 cig day^{-1} or unknown amount), physical activity (<3 MET-h per week, 3–8.9 MET-h per week, 9–17.9 MET-h per week, 18–26.9 MET-h per week, 27–41.9 MET-h per week, ⩾42 MET-h per week and missing), oral contraceptive use (current user, never user, past user 0–23 months ago, past user 24–47 months ago, past user 48–71 months ago, past user 72–95 months ago, past user 96–119 months ago, past user ⩾120 months ago and missing), frequency of multivitamin use (non-users, ⩽2 tablets $week^{-1}$, 3–5 tablets $week^{-1}$, ⩾6 tablets $week^{-1}$ and missing), intake of alcohol (no intake, <2 g day^{-1}, 2–4.9 g day^{-1}, ⩾5 g day^{-1}), coffee (<1 serving per month, 1 serving per month, 2–6 servings per week, 1 serving per day, 2–3 servings per day, ⩾4 servings per day), iron (quintiles) *trans* fatty acids (continuous), animal protein (continuous) and vegetable protein (continuous).

Table 5 Risk ratios (RR) and 95% confidence intervals (CI)[a] of ovulatory infertility associated with increasing fiber intake by 10 g day^{-1} in subgroups of the study population

Subgroup	*Cases (n)*	*Total fiber*		*Cereal fiber*	
		RR (95% CI)	P *interaction*	*RR (95% CI)*	P *interaction*
Age ⩽32 years	214	1.03 (0.79–1.34)	0.02	0.94 (0.61–1.44)	0.02
Age >32 years	224	0.82 (0.62–1.08)		0.56 (0.34–0.93)	
Cycles ⩾40 days	52	0.89 (0.69–1.16)	0.75	0.77 (0.52–1.14)	0.55
Cycles <40 days	386	0.99 (0.62–1.57)		0.60 (0.22–1.66)	
BMI <25	248	0.88 (0.65–1.19)	0.16	0.78 (0.49–1.22)	0.44
BMI ⩾25	190	0.96 (0.71–1.30)		0.65 (0.36–1.20)	
Nulliparous	208	0.75 (0.54–1.05)	0.03	0.79 (0.50–1.23)	0.39
Parous	230	1.06 (0.80–1.41)		0.66 (0.37–1.19)	

[a]Adjusted for age (continuous), calendar time (four 2-year intervals), total energy intake (continuous), body mass index (<20, 20–24.9, 25–29.9, ⩾30 and missing), parity (0, 1, ⩾2 and missing), smoking history (never, past 1–4 cig day^{-1}, past 5–14 cig day^{-1}, past 15–24 cig day^{-1}, past ⩾25 cig day^{-1} or unknown amount, current 1–4 cig day^{-1}, current 5–14 cig day^{-1}, current 15–24 cig day^{-1} and current ⩾25 cig day^{-1} or unknown amount), physical activity (<3 MET-h per wk, 3–8.9 MET-h per week, 9–17.9 MET-h per week, 18–26.9 MET-h per week, 27–41.9 MET-h per week, ⩾42 MET-h per week and missing), oral contraceptive use (current user, never user, past user 0–23 months ago, past user 24–47 months ago, past user 48–71 months ago, past user 72–95 months ago, past user 96–119 months ago, past user ⩾120 months ago and missing), frequency of multivitamin use (non-users, ⩽2 tablets $week^{-1}$, 3–5 tablets $week^{-1}$, ⩾6 tablets $week^{-1}$ and missing), intake of alcohol (no intake, <2 g day^{-1}, 2–4.9 g day^{-1}, ⩾5 g day^{-1}), coffee (<1 serving per month, 1 serving per month, 2–6 servings per week, 1 serving per day, 2–3 servings per day, ⩾4 servings per day), iron (quintiles) *trans*-fatty acids (continuous), animal protein (continuous), vegetable protein (continuous), and glycemic load (continuous).

Acknowledgements

Financial Support: The work reported in this article was supported by CA50385, the main Nurses' Health Study II grant, by the training Grant T32 DK-007703 and by the Yerby Postdoctoral Fellowship Program.

The Nurses Health Study II is supported for other specific projects by the following NIH Grants: CA55075, CA67262, AG/CA14742, CA67883, CA65725, DK52866, HL64108, HL03804.

References

Azziz R, Ehrmann D, Legro RS, Whitcomb RW, Hanley R, Fereshetian AG *et al*. (2001). Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial. *J Clin Endocrinol Metab* **86**, 1626–1632.

Brand-Miller J, Hayne S, Petocz P, Colagiuri S (2003). Low-glycemic index diets in the management of diabetes: a meta-analysis of randomized controlled trials. *Diabetes Care* **26**, 2261–2267.

Brettenthaler N, De Geyter C, Huber PR, Keller U (2004). Effect of the insulin sensitizer pioglitazone on insulin resistance, hyperandrogenism, and ovulatory dysfunction in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* **89**, 3835–3840.

Chandra A, Martinez GM, Mosher WD, Abma JC, Jones J (2005). Fertility, family planning, and reproductive health of US women: data from the 2002 national survey of family growth. *Vital Health Stat* **23**, 1–160.

Chavarro JE, Rich-Edwards JW, Rosner BA, Willett WC (2006). Iron intake and risk of ovulatory infertility. *Obstet Gynecol* **108**, 1145–1152.

Chavarro JE, Rich-Edwards JW, Rosner BA, Willett WC (2007). Dietary fatty acid intakes and the risk of ovulatory infertility. *Am J Clin Nutr* **85**, 231–237.

Chavarro JE, Rich-Edwards JW, Rosner BA, Willett WC (in press). Use of multivitamins, intake of B vitamins and risk of ovulatory infertility. *Fertil Steril*; 2007 July 9; E-pub ahead of print; doi:10.1016/j.fertnstert.2007.03.089.

Chen Y, Coulston AM, Zhou M, Hollenbeck CB, Reaven GM (1995). Why do low-fat high-carbohydrate diets accentuate postprandial lipemia in patients with niddm? *Diabetes Care* **18**, 10–16.

Coulston AM, Liu GC, Reaven GM (1983). Plasma glucose, insulin and lipid responses to high-carbohydrate low-fat diets in normal subjects. *Metabolism* **32**, 52–56.

Deuster PA, Kyle SB, Moser PB, Vigersky RA, Singh A, Schoomaker EB (1986). Nutritional intakes and status of highly trained amenorrheic and eumenorrheic women runners. *Fertil Steril* **46**, 636–643.

Douglas CC, Gower BA, Darnell BE, Ovalle F, Oster RA, Azziz R (2006a). Role of diet in the treatment of polycystic ovary syndrome. *Fertil Steril* **85**, 679–688.

Douglas CC, Norris LE, Oster RA, Darnell BE, Azziz R, Gower BA (2006b). Difference in dietary intake between women with polycystic ovary syndrome and healthy controls. *Fertil Steril* **86**, 411–417.

Dunaif A (1997). Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocr Rev* **18**, 774–800.

Ehrmann DA (2005). Polycystic ovary syndrome. *N Engl J Med* **352**, 1223–1236.

Fitzmaurice GM, Laird NM, Ware JH (2004). Marginal models: generalized estimating equations (GEE). In: Fitzmaurice GM *et al*. (ed). *Applied Longitudinal Analysis*, Wiley & Sons: Hoboken, NJ, pp 291–321.

Garg A, Bantle JP, Henry RR, Coulston AM, Griver KA, Raatz SK *et al*. (1994). Effects of varying carbohydrate content of diet in patients with non-insulin-dependent diabetes mellitus. *JAMA* **271**, 1421–1428.

Hill P, Garbaczewski L, Haley N, Wynder E (1984). Diet and follicular development. *Am J Clin Nutr* **39**, 771–777.

Hjollund NHI, Jensen TK, Bonde JPE, Henriksen NE, Andersson AM, Skakkebaek NE (1999). Is glycosylated haemoglobin a marker of fertility? A follow-up study of first-pregnancy planners. *Hum Reprod* **14**, 1478–1482.

Hull MG, Glazener CM, Kelly NJ, Conway DI, Foster PA, Hinton RA *et al*. (1985). Population study of causes, treatment, and outcome of infertility. *Br Med J* **291**, 1693–1697.

Jenkins DJ, Wolever TM, Taylor RH, Barker H, Fielden H, Baldwin JM *et al*. (1981). Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr* **34**, 362–366.

Jeppesen J, Schaaf P, Jones C, Zhou M, Chen Y, Reaven G (1997). Effects of low-fat, high-carbohydrate diets on risk factors for ischemic heart disease in postmenopausal women. *Am J Clin Nutr* **65**, 1027–1033.

Jimenez-Cruz A, Bacardi-Gascon M, Turnbull WH, Rosales-Garay P, Severino-Lugo I (2003). A flexible, low-glycemic index Mexican-style diet in overweight and obese subjects with type 2 diabetes improves metabolic parameters during a 6-week treatment period. *Diabetes Care* **26**, 1967–1970.

Kaaks R, Lukanova A (2001). Energy balance and cancer: the role of insulin and insulin-like growth factor-i. *Proc Nutr Soc* **60**, 91–106.

Lau C, Faerch K, Glumer C, Tetens I, Pedersen O, Carstensen B *et al*. (2005). Dietary glycemic index, glycemic load, fiber, simple sugars, and insulin resistance: the inter99 study. *Diabetes Care* **28**, 1397–1403.

Lefevre M, Lovejoy JC, Smith SR, DeLany JP, Champagne C, Most MM *et al*. (2005). Comparison of the acute response to meals enriched with *cis*- or *trans*-fatty acids on glucose and lipids in overweight individuals with differing fabp2 genotypes. *Metabolism* **54**, 1652–1658.

Liese AD, Schulz M, Fang F, Wolever TMS, D'Agostino Jr RB, Sparks KC *et al*. (2005). Dietary glycemic index and glycemic load, carbohydrate and fiber intake, and measures of insulin sensitivity, secretion, and adiposity in the insulin resistance atherosclerosis study. *Diabetes Care* **28**, 2832–2838.

Liu GC, Coulston AM, Reaven GM (1983). Effect of high-carbohydrate-low-fat diets on plasma glucose, insulin and lipid responses in hypertriglyceridemic humans. *Metabolism* **32**, 750–753.

Liu S, Manson JE, Stampfer MJ, Holmes MD, Hu FB, Hankinson SE *et al*. (2001). Dietary glycemic load assessed by food-frequency questionnaire in relation to plasma high-density-lipoprotein cholesterol and fasting plasma triacylglycerols in postmenopausal women. *Am J Clin Nutr* **73**, 560–566.

Liu S, Willett WC, Stampfer MJ, Hu FB, Franz M, Sampson L *et al*. (2000). A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in US women. *Am J Clin Nutr* **71**, 1455–1461.

Mayer-Davis EJ, Dhawan A, Liese AD, Teff K, Schulz M (2006). Towards understanding of glycaemic index and glycaemic load in habitual diet: associations with measures of glycaemia in the insulin resistance atherosclerosis study. *Br J Nutr* **95**, 397–405.

McKeown NM, Meigs JB, Liu S, Saltzman E, Wilson PWF, Jacques PF (2004). Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic syndrome in the Framingham offspring cohort. *Diabetes Care* **27**, 538–546.

Miller JB, Pang E, Broomhead L (1995). The glycemic index of foods containing sugars: comparison of foods with naturally-occurring v. added sugars. *Br J Nutr* **73**, 613–623.

Moghetti P, Castello R, Negri C, Tosi F, Perrone F, Caputo M *et al*. (2000). Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovarian syndrome: a randomized, double blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. *J Clin Endocrinol Metab* **85**, 139–146.

Murakami K, Sasaki S, Takahashi Y, Okubo H, Hosoi Y, Horiguchi H *et al*. (2006). Dietary glycemic index and load in relation to metabolic risk factors in Japanese female farmers with traditional dietary habits. *Am J Clin Nutr* **83**, 1161–1169.

Oh K, Hu FB, Cho E, Rexrode KM, Stampfer MJ, Manson JE *et al*. (2005). Carbohydrate intake, glycemic index, glycemic load, and dietary fiber in relation to risk of stroke in women. *Am J Epidemiol* **161**, 161–169.

Ostman EM, Frid AH, Groop LC, Bjorck IME (2006). A dietary exchange of common bread for tailored bread of low glycaemic index and rich in dietary fibre improved insulin economy in young women with impaired glucose tolerance. *Eur J Clin Nutr* **60**, 334–341.

Reichman M, Judd J, Taylor P, Nair P, Jones D, Campbell W (1992). Effect of dietary fat on length of the follicular phase of the menstrual cycle in a controlled diet setting. *J Clin Endocrinol Metab* **74**, 1171–1175.

Rich-Edwards JW, Goldman MB, Willett WC, Hunter DJ, Stampfer MJ, Colditz GA *et al*. (1994). Adolescent body mass index and ovulatory infertility. *Am J Obstet Gynecol* **171**, 171–177.

Rich-Edwards JW, Spiegelman D, Garland M, Hertzmark E, Hunter DJ, Colditz GA *et al*. (2002). Physical activity, body mass index, and ovulatory disorder infertility. *Epidemiology* **13**, 184–190.

Rizkalla SW, Taghrid L, Laromiguiere M, Huet D, Boillot J, Rigoir A *et al*. (2004). Improved plasma glucose control, whole-body glucose utilization, and lipid profile on a low-glycemic index diet in type 2 diabetic men: a randomized controlled trial. *Diabetes Care* **27**, 1866–1872.

Salmeron J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willett WC (1997). Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. *J Am Med Assoc* **277**, 472–477.

Schulze MB, Liu S, Rimm EB, Manson JE, Willett WC, Hu FB (2004). Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women. *Am J Clin Nutr* **80**, 348–356.

Snow RC, Schneider JL, Barbieri RL (1990). High fiber and low saturated fat intake among oligomenorrheic undergraduates. *Fertil Steril* **54**, 632–637.

The Diabetes Prevention Program Research Group (2005). Role of insulin secretion and sensitivity in the evolution of type 2 diabetes in the diabetes prevention program: effects of lifestyle intervention and metformin. *Diabetes* **54**, 2404–2414.

University of Sydney (Australia). Online glycemic index database. http://www.glycemicindex.com (accessed 2002).

Vrbikova J, Bendlova B, Hill M, Vankova M, Vondra K, Starka L (2002). Insulin sensitivity and β-cell function in women with polycystic ovary syndrome. *Diabetes Care* **25**, 1217–1222.

Willett WC, Stampfer MJ (1998). Chapter 11: implications of total energy intake for epidemiologic analyses. In: Willett WC (ed). *Nutritional Epidemiology*, 2nd edn. Oxford University Press: New York, pp 273–301.

Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J *et al*. (1985). Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* **122**, 51–65.