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High dietary choline and betaine intake is associated with low insulin resistance in the Newfoundland population

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ABSTRACT

Objective: Dietary betaine supplement could ameliorate insulin resistance (IR) in animals, but no data are available for choline. Reports on humans are rare. The aim of this study was to investigate the association between dietary choline and betaine intake and IR in humans.

Methods: We assessed 2394 adults from the CODING (Complex Diseases in the Newfoundland population: Environment and Genetics) study. Intake of dietary choline and betaine was evaluated from the Willett Food Frequency Questionnaire. IR was estimated by homeostatic model assessment (HOMA-IR) and the quantitative insulin-sensitivity check index (QUICKI). Partial correlation analysis was used to determine the correlations of dietary choline and betaine intake with IR adjusted for major confounding factors.

Results: Dietary choline and betaine intake was inversely correlated with levels of fasting glucose and insulin, HOMA-IR, HOMA-β ($r = -0.08$ to -0.27 for choline and $r = -0.06$ to -0.16 for betaine; $P < 0.05$) and positively related to QUICKI ($r = 0.16$–0.25 for choline and $r = 0.11$–0.16 for betaine; $P < 0.01$) in both sexes after controlling for age, total calorie intake, and physical activity level. The significant associations disappeared in men after percent trunk fat was added as a confounding factor. Furthermore, individuals with the highest tertile of dietary choline and betaine intake had the lowest IR severity. Dietary choline and betaine intake, however, was the lowest in the high IR group, intermediate in the medium group, and the highest in the low IR group.

Conclusion: This study demonstrated that higher intake of dietary choline and betaine is associated with lower IR in the general population.

Introduction

Type 2 diabetes (T2DM) comprises 90% of all diabetic cases and has become a major public health problem globally [1]. The prevalence of T2DM is expected to reach >438 million people globally by the year 2030 [1]. As a complex disease, the mechanism of T2DM is still not completely understood. Possible reasons include age, genetics and lifestyle (physical inactivity and unhealthy food consumption) factors [2], with insulin resistance (IR) playing the crucial role in the pathogenesis of T2DM [3]. To date, although a panel of drugs is used to treat diabetes currently, such as metformin, insulin analog, α-glucosidase inhibitor, and glucagon-like peptide-1 (GLP-1) receptor agonists [4], simple lifestyle modifications (such as increased physical activity levels, moderate weight loss, eating behavior modifications, or nutrition composition) have been shown to attenuate the onset of T2DM [2,5]. Currently, studies have discussed the association of some dietary nutrients intakes with IR or T2DM in the general population [6–9].

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Choline and betaine are metabolically related quaternary ammonium compounds, which are obtained from diet or by synthesis de novo. Both of them are abundant in a wide variety of foods. Dietary sources of choline are mainly eggs, beef, pork, liver, seafood, and milk, whereas betaine is obtained from grains, cereal, beets, and spinach [10–12]. Choline is an essential nutrient and plays important roles in neurotransmitter synthesis, cell-membrane signaling, lipid transport, and methyl-group metabolism by serving as a precursor for acetylcholine, phospholipids, lipoproteins, and the methyl donor betaine [12,13]. Betaine, a derivative of choline, serves as a compatible osmolyte in cells and a methyl donor in many pathways, including the homocysteine methylation [14].

Studies have suggested that choline and betaine play important roles in the prevention of various diseases [12,15–17]. Evidence linking choline, betaine, and IR are limited and largely performed in animal experiments. Reports have shown that supplementation with dietary betaine could improve IR in mice [18,19]. Choline depletion during high-fat diet-fed or ob/ob mice could attenuate IR [20,21]. To our knowledge, there is no data available on the effect of choline supplement. In humans, studies regarding the effect of choline and betaine on IR are rare. Serum choline, but not betaine levels, were reported to be inversely associated with the risk for T2DM [22]. Decreased serum choline levels serve as possible predictors of impaired glucose tolerance (IGT) and IR risks in the prediabetic state [23]. It has been found that urinary excretion of betaine is increased in patients with diabetes [24,25]. To the best of our knowledge, no published study has examined the effect of dietary choline or betaine on IR in humans.

We hypothesize that the intake of dietary choline and betaine is inversely associated with IR. Therefore, the present study was designed to investigate the association of dietary choline and betaine intake with IR in the general Newfoundland population, taking into consideration the major confounding factors.

Methods

Study population

All participants were from the CODING (Complex Diseases in the Newfoundland population: Environment and Genetics) study [26,27]. The Newfoundland population in the present CODING study is well known as a genetically homogeneous population in North America. The population consists of English, Irish, Scottish, and a small proportion of French origins. Inclusion criteria were as follows: ≥19 y of age; at least a third-generation Newfoundlander; and physically able to travel to our research center and complete all questionnaires and measurements. Additionally, women were not breast feeding or pregnant at the time of the study. We recruited 3214 participants, 820 of whom had incomplete data and were excluded. Therefore, 2394 eligible individuals (1783 women and 611 men) were included in the present study. There were no significant differences on demographic variables between those excluded and included.

Participants provided written and informed consent and the study received ethical approval from the Health Research Ethics Authority (HREA), Memorial University, St. John's, Newfoundland, Canada, with Project Identification Code #10.33.

Anthropometric and body composition measurements

Anthropometrics and body composition measurements were collected after a 12-h fast. After urinating to empty their bladders, participants were weighed to the nearest 0.1 kg in standard hospital gowns using a platform manual scale balance (Health O Meter, Bridgeview, IL, USA). Standing height was measured using a fixed stadiometer to the nearest 0.1 cm. Body mass index (BMI; kg/m^2) was calculated from weight and height in kilograms per square meter. Waist circumference (WC) was measured midway between the iliac crest and the lower rib.

Dual energy x-ray absorptiometry (DXA; Lunar Prodigy; GE Medical Systems, Madison, WI, USA) was used for the measurement of total percent body fat (%BF) and percent trunk fat (%TF). The enCORE (Ver 12.2, 2008, GE Medical Systems) software package was used for DXA data analysis. Quality assurance was performed on the DXA scanner daily and the typical coefficient of variation was 1.3% during the study period [27].

Lifestyle and dietary assessment

Information regarding participants' lifestyles was collected through a self-administered screening questionnaire. The questions were related to demographic characteristics (age, sex, and family origin), disease status, smoking status, alcohol consumption, and medication use. Women completed an additional questionnaire regarding their menopausal status. Physical activity patterns were measured using the Ability of the Atherosclerosis Risk in Communities/Baecke Questionnaire, including work, sports, and leisure time activity indexes [28].

Dietary intake of each participant was assessed using a 124-item semi-quantitative Willett Food Frequency Questionnaire (FFQ) [29,30], which has been validated in the Newfoundland population [31]. Any calorie intake that was too high or too low defined by 3 SDs outside the mean was not included. The Willett FFQ obtained from participants the number of weekly servings consumed of common food items over the past year. Daily intake for each food item consumed was entered into NutriBase Clinical Nutrition Manager (version 8.2.0; CybersoftInc, Phoenix, AZ, USA) software package, and the total daily intake of calorie (kcal/d), choline (mg/d), and betaine (mg/d) for each individual was computed automatically [29]. Daily dietary choline and betaine intake per kilogram body weight (mg/kg) was calculated.

Biochemical measurements

Venous blood samples were collected from all participants after a 12-h fasting period. Serum samples were isolated and stored at −80°C for subsequent analysis. Fasting plasma glucose (FPG) were measured on an Lx20 analyzer (Beckman Coulter Inc., Fullerton, CA, USA) using Synchron reagents. Fasting insulin (FINS) was measured on an Immulite Immunoassay (Siemans Healthcare GmbH, Erlangen, Germany) analyzer. IR and β-cell function were determined with the homeostasis model assessment (HOMA-IR and HOMA- β) [32]:

$$\text{HOMA} - \text{IR} = \frac{(\text{FINS [mU/L]} \times \text{FPG [mmol/L]})}{22.5}$$

$$\text{HOMA} - \beta = \frac{(20 \times \text{FINS [mU/L]})}{(\text{FPG [mmol/L]} - 3.5)}$$

Quantitative insulin-sensitivity check index (QUICKI) was another index used for the measurement of insulin sensitivity [33]. It is determined by the following mathematical equation [33]:

$$\text{QUICKI} = \frac{1}{(\log \text{FINS [mU/L]} + \log [\text{FPG (mmol/L)} \times 18.0182])}$$

Statistical analysis

All data are presented as means ± SD. FINS, HOMA-IR, HOMA-β, calorie intake, dietary intake of choline and betaine were log-transformed to normalize the data distributions to perform effective statistical analysis. Differences in anthropometrics, body compositions, dietary intake, and biochemical measurements between women and men were assessed with independent Student's *t* test.

Statistical interaction between dietary intake of choline and betaine and sex on the main outcomes was tested by analysis of covariance (ANCOVA). Pearson correlation analyses were used to examine the relationship between various potential factors that may have an effect on insulin sensitivity. Partial correlation analysis controlling for age, total calorie intake, physical activity level, and %TF was used to find the correlations of dietary choline and betaine intake with FPG, FINS, HOMA-IR, HOMA-β, and QUICKI. We took %TF as confounding factor, rather than other obesity or fat percentage indexes because it had the highest correlation coefficient with IR indexes during Pearson correlation analysis in both sexes.

Furthermore, participants were divided into tertiles (low, medium, and high) based on daily dietary choline or betaine intake expressed in mg/kg. Differences of FPG, FINS, HOMA-IR, HOMA-β, and QUICKI between groups were assessed with ANCOVA controlling for age, total calorie intake, physical activity level, %TF in both women and men. Finally, participants were divided into tertiles (low, medium, and high) based on grade of IR assessed by HOMA-IR. Differences of dietary choline and betaine intake between groups were assessed with ANCOVA. Age, total calorie intake, physical activity level, and %TF were taken as confounding factors in both sexes.

All statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). All tests were two-sided; $P < 0.05$ was considered statistically significant.

Table 1
Physical, biochemical, and dietary intake characteristics of the study participants*

	Entire cohort (N = 2394)†	Women (n = 1783)†	Men (n = 611)†	*P* value‡
Age, y	43.40 ± 12.81	44.08 ± 12.32	41.71 ± 13.93	<0.001
Weight, kg	75.19 ± 16.70	70.47 ± 13.82	88.10 ± 15.49	<0.001
BMI, kg/m^2	27.18 ± 5.13	26.75 ± 5.10	28.26 ± 4.55	<0.001
WC, cm	93.07 ± 14.16	90.97 ± 13.86	99.18 ± 12.87	<0.001
%TF	37.59 ± 9.28	39.66 ± 8.45	31.81 ± 8.83	<0.001
%BF	35.15 ± 9.05	38.22 ± 7.37	26.40 ± 7.42	<0.001
FPG, mmol/L	5.15 ± 0.85	5.08 ± 0.77	5.37 ± 0.92	<0.001
FINS, pmol/L	69.41 ± 45.64	66.79 ± 42.31	74.18 ± 48.50	<0.001
HOMA-IR	2.38 ± 1.98	2.25 ± 1.78	2.57 ± 1.85	<0.001
HOMA-β	131.87 ± 102.44	133.67 ± 100.93	124.71 ± 97.93	<0.001
QUICKI	0.35 ± 0.03	0.35 ± 0.03	0.34 ± 0.03	<0.001
Physical activity	8.12 ± 1.52	8.05 ± 1.48	8.38 ± 1.58	<0.001
Calorie intake, kcal/d	1953.98 ± 887.91	1859.92 ± 812.85	2207.11 ± 962.20	<0.001
Choline, mg/d	304.99 ± 237.33	287.65 ± 210.88	346.69 ± 274.13	<0.001
Choline, mg/kg/d	4.21 ± 3.43	4.24 ± 3.35	4.03 ± 3.17	0.012
Betaine, mg/d	106.54 ± 87.90	104.50 ± 82.69	111.15 ± 97.26	0.380
Betaine, mg/kg/d	1.48 ± 1.27	1.54 ± 1.26	1.30 ± 1.19	<0.001

%BF, percent body fat; BMI, body mass index; FINS, fasting insulin; FPG, fasting plasma glucose; HOMA-IR, homeostatic model assessment for insulin resistance; QUICKI, quantitative insulin-sensitivity check index; %TF, percent trunk fat; WC, waist circumference
All the data reported are original, whereas FINS, HOMA-IR, HOMA-β, calorie intake, dietary choline and betaine intake were log-transformed to normalize the data distributions during statistical analysis
* All values are mean ± SD.
† Sample size range for study group (see Participants).
‡ Significant differences between female and male groups, based on independence sample. Student's *t test*, statistical significance was set to $P < 0.05$.

Statistical powers were >80% ($\alpha = 0.05$, two-sided), and the sample sizes were large enough in all the tests.

Results

Physical and dietary characteristics of participants

Clinical and dietary characteristics of the participants are presented in Table 1. The women were on average 2.4 y older than the men. Weight, BMI, WC, FPG, FINS, HOMA-IR, and physical activity levels were significantly higher in men than in women; however, %TF, %BF, HOMA-β, and QUICKI were significantly higher in women ($P < 0.001$ for all). In terms of dietary intake, the men had a significantly higher calorie and choline intake (mg/d) than women ($P < 0.001$) and there was no significant difference for dietary betaine (mg/d) between the sexes. When daily dietary choline and betaine intake was expressed by per kilogram body weight (mg/kg), women consumed significantly more choline and betaine than the men ($P < 0.05$).

Correlations between dietary choline or betaine intake and IR measurements

Significant interactions between dietary choline and betaine intake and sex on FPG, FINS, HOMA-IR, HOMA-β, and QUICKI were found ($P < 0.05$ for all). The correlations of dietary choline and betaine intake with IR indexes are presented in Table 2. Daily dietary choline and betaine intake (mg/kg) were significantly negatively associated with FPG, FINS, HOMA-IR, and HOMA-β ($P < 0.05$ for all), and positively associated with QUICKI ($P < 0.01$) in both sexes after adjusting for age, total calorie intake, and physical activity levels. When %TF was added to the confounding factors, the correlations remained significant in women except for FPG, but all disappeared in the men.

Furthermore, to overcome the possible influence of smoking, medication use, drinking, and menopausal status, participants were divided into subgroups according to smoking status (yes or no), medication use (yes or no), and alcohol consumption (yes or no). Women were further divided into pre- or postmenopausal groups. After separating the study sample to account for these covariates, the associations remained significant (Supplementary Tables 1–4).

Comparison of IR indexes in different groups of dietary choline or betaine intake

When participants were grouped into tertiles according to daily dietary choline intake (mg/kg) (Table 3), FINS, HOMA-IR, and HOMA-β presented a significant dose-dependent decline, whereas QUICKI presented a dose-dependent increasing trend with the increase of dietary choline intake in both men and women after controlling for age, total calorie intake, and physical activity levels ($P < 0.05$ for all). For every 1 mg/kg increase in daily dietary choline intake, on average HOMA-IR, HOMA-β, and

Table 2
Partial correlations between dietary choline, betaine (mg/kg/d) intakes and IR indexes

	Female (n = 1783)		Male (n = 611)	
	r1* (*P* value)†	r2 (*P* value)†	r1 (*P* value)†	r2 (*P* value)†
Daily choline intake, mg/kg				
FBG	−0.10 (0.002)	−0.03 (0.238)	−0.08 (0.026)	0.02 (0.342)
FINS	−0.27 (0.000)	−0.15 (0.000)	−0.14 (0.000)	−0.03 (0.486)
HOMA-IR	−0.26 (0.000)	−0.14 (0.000)	−0.14 (0.000)	−0.03 (0.435)
HOMA-β	−0.20 (0.000)	−0.12 (0.000)	−0.11 (0.009)	−0.01 (0.899)
QUICKI	0.25 (0.000)	0.13 (0.000)	0.16 (0.009)	0.04 (0.312)
Daily betaine intake, mg/kg				
FPG	−0.06 (0.017)	−0.02 (0.542)	−0.078 (0.028)	0.01 (0.140)
FINS	−0.16 (0.000)	−0.08 (0.000)	−0.109 (0.007)	−0.02 (0.658)
HOMA-IR	−0.16 (0.000)	−0.08 (0.000)	−0.091 (0.024)	0.00 (0.991)
HOMA-β	−0.11 (0.000)	−0.06 (0.012)	−0.130 (0.001)	−0.06 (0.171)
QUICKI	0.16 (0.000)	0.08 (0.006)	0.106 (0.008)	0.01 (0.729)

FINS, fasting insulin; FPG, fasting plasma glucose; HOMA-IR, homeostatic model assessment; IR, insulin resistance; QUICKI, quantitative insulin-sensitivity check index
* r1: partial correlations controlling for age, total calorie intake, physical activity level; r2: partial correlations controlling for age, total calorie intake, physical activity level and percent trunk fat.
† Statistical significance was set to $P < 0.05$.

Table 3
IR indexes according to the amount of daily dietary choline (mg/kg) intake*

	Low†	Medium†	High†	P1‡	P2
Female					
n	595	594	594		
FBG	5.19 ± 0.85	5.04 ± 0.68	5.01 ± 0.77	0.000	0.015
FINS	79.71 ± 50.66	64.06 ± 37.35	57.23 ± 34.70	0.000	0.000
HOMA-IR	2.73 ± 2.04	2.12 ± 1.59	1.92 ± 1.64	0.000	0.000
HOMA-β	151.97 ± 128.95	129.24 ± 2.63	120.15 ± 79.88	0.000	0.000
QUICKI	0.34 ± 0.03	0.35 ± 0.03	0.36 ± 0.03	0.000	0.000
Daily choline, mg/kg	2.10 ± 0.54 (0.36–2.84)	3.51 ± 0.41 (2.85–4.31)	7.09 ± 4.49 (4.32–51.27)		
Male					
n	204	204	203		
FPG	5.52 ± 0.93	5.36 ± 1.10	5.25 ± 0.68	0.149	0.061
FINS	92.13 ± 55.81	68.69 ± 43.99	63.61 ± 44.88	0.000	0.007
HOMA-IR	3.24 ± 2.25	2.32 ± 1.49	2.22 ± 1.77	0.000	0.004
HOMA-β	141.50 ± 94.12	117.83 ± 77.77	116.68 ± 118.54	0.018	0.147
QUICKI	0.33 ± 0.03	0.35 ± 0.035	0.35 ± 0.035	0.000	0.009
Daily choline, mg/kg	1.90 ± 0.50 (0.51–2.59)	3.28 ± 0.44 (2.60–4.08)	6.89 ± 4.07 (4.09–35.90)		

ANCOVA, analysis of covariance; FINS, fasting insulin; FPG, fasting plasma glucose; HOMA-IR, homeostatic model assessment; IR, insulin resistance; QUICKI, quantitative insulin-sensitivity check index
Statistical significance was set to $P < 0.05$
* All values are mean ± SD.
† Participants were divided into low, medium, and high daily choline intake groups based on mg/kg.
‡ *P1*: Significant differences between different dietary choline intake amount groups. Data were assessed with ANCOVA controlling for age, total calorie intake, physical activity level; *P2*: significant differences between different dietary choline intake amount groups. Data were assessed using ANCOVA controlling for age, total calorie intake, physical activity level and percent trunk fat.

QUICKI indexes changed by −0.23, −9.28, and 0.004 in women, and −0.14, −4.35, and 0.003 in men, respectively. When %TF was added to the confounding factors, the dose-dependent variations were still significant except for HOMA-β in men. FPG presented a significant dose-dependent decline only in women.

When participants were grouped into tertiles according to daily dietary betaine intake (mg/kg; Table 4), FINS, HOMA-IR, and HOMA-β presented a significant dose-dependent decline, whereas QUICKI present a dose-dependent increasing trend with the increase of dietary betaine intake in both men and women after controlling for age, total calorie intake, and physical activity levels ($P < 0.05$ for all). For every 1 mg/kg increase in daily dietary betaine intake, on average HOMA-IR, HOMA-β, and QUICK indexes changed by −0.29, −10.11, and 0.005 in women, and −0.16, −8.07, and 0.004 in men, respectively. When %TF was added to the confounding factors, the dose-dependent variations were still significant in women ($P < 0.05$ for all) but disappeared in men. FPG did not present a significant dose-dependent trend.

Comparison of dietary choline and betaine intake in groups with different IR status

Participants were divided by sex into tertiles (low, medium, and high) based on HOMA-IR. As shown in Table 5, after controlling for confounding factors, dietary choline intake and betaine intake were not statistically different among groups,

Table 4
IR indexes according to daily dietary betaine (mg/kg) intake*

	Low†	Medium†	High†	P1‡	P2
Female					
n	595	594	594		
Glucose	5.13 ± 0.72	5.05 ± 0.72	5.06 ± 0.87	0.191	0.486
Insulin	77.45 ± 49.53	63.66 ± 39.07	59.91 ± 35.81	0.000	0.000
HOMA-IR	2.64 ± 2.11	2.14 ± 1.70	1.99 ± 1.47	0.000	0.000
HOMA-β	148.43 ± 121.78	128.74 ± 84.54	124.22 ± 89.88	0.000	0.004
QUICKI	0.343 ± 0.032	0.353 ± 0.0315	0.357 ± 0.032	0.000	0.001
Daily betaine, mg/kg	0.47 ± 0.21 (0.03–0.84)	1.2 ± 0.25 (0.85–1.68)	2.9 ± 1.26 (1.69–13.73)		
Male					
n	204	204	203		
Glucose	5.42 ± 0.91	5.35 ± 0.80	5.35 ± 1.05	0.102	0.052
Insulin	87.13 ± 55.07	73.28 ± 48.79	64.14 ± 43.03	0.009	0.190
HOMA-IR	2.93 ± 2.00	2.58 ± 1.99	2.27 ± 1.70	0.023	0.302
HOMA-β	137.95 ± 83.36	127.54 ± 118.93	110.63 ± 88.57	0.012	0.174
QUICKI	0.336 ± 0.029	0.345 ± 0.033	0.352 ± 0.034	0.015	0.241
Daily betaine, mg/kg	0.40 ± 0.18 (0.04–0.69)	0.95 ± 0.16 (0.70–1.27)	2.54 ± 1.32 (1.28–10.99)		

ANCOVA, analysis of covariance; FINS, fasting insulin; FPG, fasting plasma glucose; HOMA-IR, homeostatic model assessment; IR, insulin resistance; QUICKI, quantitative insulin-sensitivity check index
Statistical significance was set to $P < 0.05$.
* All values are mean ± SD.
† Participants were divided into low, medium, and high daily betaine intake groups based on mg/kg.
‡ *P1*: Significant differences between different dietary betaine intake amount groups. Data were assessed with ANCOVA controlling for age, total calorie intake, physical activity level; *P2*: significant differences between different dietary betaine intake amount groups. Data were assessed ANCOVA controlling for age, total calorie intake, physical activity level and %TF.

Table 5
Comparison of dietary choline and betaine intakes in different IR status*

HOMI-IR	Low†	Medium†	High†	*P*1‡	*P*2
Female					
n	595	594	594		
HOMA-IR	0.41–1.41	1.42–2.29	2.30–24.07		
Choline, mg/d	301.62 ± 239.06	284.82 ± 169.91	275.54 ± 212.72	0.049	0.076
Choline, mg/kg/d	4.82 ± 4.13	4.28 ± 2.85	3.60 ± 2.71	0.000	0.000
Betaine, mg/d	108.38 ± 80.72	106.50 ± 85.45	99.68 ± 83.81	0.082	0.329
Betaine, mg/kg/d	1.72 ± 1.35	1.59 ± 1.30	1.30 ± 1.10	0.000	0.006
Male					
n	204	204	203		
HOMA-IR	0.40–1.61	1.62–2.76	2.77–28.66		
Choline, mg/d	395.71 ± 340.83	322.65 ± 197.62	320.14 ± 252.24	0.318	0.332
Choline, mg/kg/d	4.90 ± 4.14	3.77 ± 2.31	3.37 ± 2.48	0.010	0.853
Betaine, mg/d	116.91 ± 95.82	107.16 ± 84.79	108.49 ± 108.12	0.898	0.776
Betaine, mg/kg/d	1.46 ± 1.28	1.25 ± 1.01	1.17 ± 1.24	0.218	0.964

ANCOVA, analysis of covariance; HOMA-IR, homeostatic model assessment; IR, insulin resistance
Statistical significance was set to $P < 0.05$
* All values are mean ± SDs.
† Participants were divided into low, medium, and high IR groups based on HOMA-IR.
‡ *P*1: Significant differences between different IR groups. Data were assessed with ANCOVA controlling for age, total calorie intake, physical activity level; *P*2: Significant differences between different IR groups. Data were assessed ANCOVA controlling for age, total calorie intake, physical activity level and percent trunk fat.

except for choline intake after controlling for age, total calorie intake, and physical activity levels. When dietary intake of choline and betaine was expressed in mg/kg daily, both declined significantly from low to high HOMA-IR groups in women ($P_{trend} < 0.05$) even after controlling confounding factors. In men, no more significance remained after all confounding factors were adjusted.

Moreover, to avoid the potential interference of diabetes on the relationship, all analyses were repeated after volunteers who had FPG >7 mmol/L or reported to have diabetes were excluded. All findings remained significant (Supplementary Tables 5–8).

Discussion

To the best of our knowledge, this is the first study to evaluate the association of dietary choline and betaine intake with IR, with the most comprehensive controls for major confounding factors, in the general population. The core finding in the present study is the discovery that dietary choline and betaine intake was negatively correlated with IR in the large population-based CODING study. The results were consistent using either HOMA-IR or QUICKI methods. More importantly, the associations were independent of age, sex, total calorie intake, and physical activity levels. The second finding is the sex difference. The association was more pronounced in women than in men. The negative association between dietary choline and betaine intake and IR seemed partly mediated by adiposity, especially in men.

The strength of the present study lies in the large population-based study and the systematic control of major confounding factors. IR is a complex pathophysiological condition with numerous factors involved in its development. Correctly identifying, measuring, and controlling these factors in analyses are critical to reveal true and reliable associations. IR variations between men and women make sex the first factor needed to be controlled [34]. Age is an important factor that is negatively associated with insulin sensitivity [35]. Total calorie intake is a critical factor in maintaining energy balance and is generally negatively related with insulin sensitivity [36]. Physical activity level is likely the most important nondietary factor that can potently influence insulin sensitivity and glucose metabolism [37]. In the present study, all these factors were properly controlled and the negative relationship between dietary choline and betaine intake and IR remains significant. Obesity is another well-known condition that can place individuals at a significantly elevated risk for impaired insulin action [35]. After %TF was adjusted, the correlation coefficient decreased in both women and men. In previous studies, choline and betaine were shown to be negatively associated with body fat in animals and humans [38–40]. Taking body fat as a confounding factor may overadjust the correlation between the main factors. Otherwise, the correlation of dietary choline and betaine with IR may partly dependent on their effect on attenuating obesity. Furthermore, when excluding the potential interference of diabetes on the relationships, all findings remained significant. Moreover, we took menopause, smoking, medication, and alcohol consumption into consideration. Menopause is an important factor related to changes in sexual hormones, weight gain, and IR severity in women [41,42]. The relationship between dietary choline and betaine intake and IR was profound in both pre- and postmenopausal women. Smoking status, medication use, and alcohol consumption are recognized as potentially covariates that may affect energy intake and IR [43–45]. The associations remained significant after all these covariates were controlled.

The association between dietary betaine and IR found in the present study was consistent with what was discovered in animals. In mice, dietary betaine supplementary could improve IR [18,19]. The effect of dietary choline might be more complex and there is no direct evidence. In humans, serum choline levels were inversely associated with the risks for T2DM [22] and decreased serum choline levels were suggested to serve as possible predictors for the evaluation of IGT and IR risks in the prediabetic state [23]. Data regarding the relationship between the level of dietary and the level of serum choline and betaine is scarce, and it seems to be positive [46,47]. However, in high-fat diet-induced or ob/ob obese mice, choline depletion amplified liver fat accumulation but attenuated IR compared with choline supplementation [20,21]. The authors explained that choline deficiency may prevent potentially toxic free fatty acids toward innocuous storage of triacylglycerol in liver. However, the condition of choline depletion is hardly seen in humans. As reported, long-term fat accumulation in the liver plays a critical role in the pathogenesis of hepatic IR [48]. Furthermore, oxidative stress, which is highly associated with IR [49], was reported to be increased in choline-deficient diet-induced hepatic steatosis rats

[50,51]. Until now, there is no large study available focusing on dietary choline and betaine intake and IR in general population. The present study filled the knowledge gap, and revealed that more dietary choline and betaine intake was associated with low IR, suggesting a beneficial effect of choline and betaine on IR.

The daily dietary intake of choline was about 4 mg/kg (350 mg/d for men and 290 mg/d for women) in our study, which did not reach the daily adequate intake (AI) set for choline (7 mg/kg) by the US Institute of Medicine's Food and Nutrition Board in 1998 [52]. However, the setting of AI for choline was based on prevention of liver damage in adult [52], and a new report in 2016 also found that dietary choline intake in the United States was lower than the recommend AI [11], which is consistent with the present study. The daily dietary betaine intake in the present study was about 1.5 mg/kg (110 mg/d for men and 104 mg/d for women). Currently, a recommended daily intake has not been established for betaine, but the recently estimated dietary intake ranges from 100 to 300 mg/d [12,14], which is similar with the result from the present study.

The exact mechanisms by which choline and betaine improve IR are unclear, although several mechanisms may partly explain the association. Choline supplementation could alleviate inflammation and suppress oxidative stress [53,54], which play important roles in the development of IR [49]. Choline can be metabolized to betaine and plays a role in altering insulin sensitivity. Betaine treatment increases hepatic insulin receptor substrate 1 phosphorylation and results in the improvement of downstream signaling pathways for gluconeogenesis and glycogen synthesis [18]. Betaine supplementation could enhance insulin sensitivity in adipose tissue as shown by improving extracellular signal regulated kinases 1/2 and protein kinase B activations [19]. Dietary betaine may improve insulin sensitivity by decreasing inflammation in adipose tissue by decreasing the expression of inflammation biomarkers, such as tumor necrosis factor-α, interleukin-6 [54,55].

Although we provided convincing results regarding the beneficial effects of choline and betaine on IR, the present study is not without limitations. The use of an FFQ to evaluate patterns of dietary intake raises the possibility of recall bias by participants [30]. However, this questionnaire has been repeatedly validated in many populations. It is the most frequently used questionnaire for the assessment of dietary intake at the population level and has been successfully used in a number of studies in Canada, including the Newfoundland population [29,31,56,57]. Thus, we consider the Willett FFQ to be a reasonable method for the assessment of dietary intake patterns in the present study. Moreover, other self-reported measures, such as physical activity levels also might have potential bias. Another weakness of this study is the cross-sectional study design. We were unable to determine whether low IR was a consequence of high choline and betaine intake based on the available data from a cross-sectional designed study. Direct intervention studies or clinical trials with reasonable sample sizes and well-matched participants between treatment and control groups are warranted. Furthermore, although multiple factors, including sociodemographic characteristics, lifestyle habits, physical activities, energy intake, and %TF were comprehensively adjusted in the present analysis, residual confounding of the results from genetic factors, different categories of medications, serum lipid profile, and unknown or poorly measured determinants of the dependent variables could not be completely ruled out. Finally, although HOMA-IR and QUICKI are widely accepted as reasonable measures of IR, the hyperinsulinemic-euglycemic clamp technique is considered a more accurate method for measuring IR [58].

Conclusion

We have shown, to our knowledge for the first time, that higher dietary choline or betaine intake was significantly associated with lower IR in a Newfoundland population. The association was stronger in women than in men. Additionally, this favorable association between dietary choline and betaine and IR was independent of age, total calorie intake, physical activity levels, and trunk fat. The present results indicate that higher dietary choline and betaine intake may improve IR.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.nut.2016.08.005.

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