Dietary Fibre, Insulin and Breast Tissue Composition at Age 15-18: A Mediation Analysis.

Lisa Martin
University Health Network
Sudipta Saha
University Health Network
Linda Linton
University Health Network
Monica Taylor
University Health Network
Jie Zhu
University Health Network
Sofia Chavez
Sunnybrook Health Sciences Centre
Greg Stanisz
Sunnybrook Health Sciences Centre
Salomon Minkin
University Health Network
Norman F Boyd (boyd@uhnres.utoronto.ca)
Campbell Family Instiute for Cancer Research https://orcid.org/0000-0002-6861-6835

Research article

Keywords: Dietary fiber, fasting insulin, breast tissue composition, mediation

Posted Date: March 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-312077/v1

Abstract

Background. Risk of breast cancer in adult life is influenced by body size and height in childhood. The mechanisms responsible for these associations are currently unknown. The present research was carried out to determine if, at age 15-18, measures of dietary intake were associated with body size, hormones and growth factors, and with variations in breast tissue composition that in adult life are strongly associated with risk of breast cancer.

Methods. We carried out a cross-sectional study in 766 healthy young Caucasian women aged 15-18. Percent breast water (PBW), total breast water and fat were measured by magnetic resonance (MR). Dietary intake at age 15-18 was assessed using a validated food frequency questionnaire. We also measured height, weight, skin-fold thicknesses and waist-to-hip ratio, and obtained fasting blood to assay glucose and insulin, and calculated an estimate of insulin sensitivity (HOMA2-S).

Results. After adjustment for age, measures of body size, and energy intake, dietary fiber (insoluble and total fiber) was associated positively with PBW with the HOMA2-S estimate of insulin sensitivity, and inversely with fasting serum insulin. Insulin sensitivity was also significantly and positively associated with PBW and inversely with total breast fat. The association of dietary fiber with breast tissue composition was no longer statistically significant after adjustment for fasting insulin or insulin sensitivity.

Conclusions. These results show an association of dietary fiber with breast tissue measures and suggest that this association may be mediated, at least in part, by insulin or insulin sensitivity. These findings suggest a potential approach to breast cancer prevention in early life.

Introduction

In childhood and adolescence, greater height, lower weight or body mass index, and leaner body type are in later life associated with a higher risk of breast cancer[1]. The mechanisms that underlie these associations are currently unknown[2]. In adult life, variations in breast tissue composition, referred to as “percent mammographic density” (PMD), have been shown to be a strong risk factor for breast cancer[3,4], and in premenopausal women, variations in PMD have been associated with variations in height and weight [5]. These findings suggest that variations in breast tissue composition in adult life may be in part the result of factors in early life that influence both body size and breast tissue composition.

The present research was carried out to determine if dietary intake at age 15-18, was associated with measures of body size, hormones and growth factors and with variations in breast tissue composition assessed by magnetic resonance (MR). Breast water determined by MR, and the risk factor PMD, both reflect breast fibro-glandular tissue and are strongly correlated [6-8].

Methods

A. General method. We carried out a cross-sectional observational study in which we recruited young women aged 15-18 years. Breast water and fat in participants were measured with MR, and anthropometric variables were measured. Diet was assessed by food frequency questionnaire, and glucose and insulin were assayed in fasting serum.

B. Recruitment of subjects. Recruitment of young women was from Toronto high schools, and family practices. Recruitment took place in two phases, Phase 1 between 2003 and 2007 and Phase 2 between 2010 and 2015. Subjects in Phase 1 have been included in a previous publication [8]. Ethics approvals were obtained from the University Health Network, Sunnybrook Hospital, and Women’s College Hospital (all in Toronto) and from the Toronto District School Board, the Toronto Catholic District School Board, the York Region District School Board and the York Catholic District School Board.

1. School recruitment. With the consent of school principals we contacted their health and/or science departments, which were asked to agree to our approaching their students in their classrooms, to present a cancer educational talk and introduce the research study. Eligible students interested in participating were given an information package to take home that described the study and included consent forms for both mother and daughter. On receipt of written consent from both mother and daughter we contacted them to set up appointments.

2. Family practice recruitment. Participating family practices contacted young women aged 15-18 on their patient lists, introduced the study and invited their participation. Those interested were mailed the same information package and consent forms that were used

in schools.

3. Inclusion and exclusion of subjects. Subjects were excluded if they had not established menses, had used oral contraceptives within the previous 6 months, had been pregnant, had breast implants, augmentation or reduction mammoplasty, or previous breast cancer. To avoid population stratification, we recruited only subjects who by self-report were white Caucasians. Exclusion criteria for the safe use of MR, included recent breast surgery, metal implants of any sort, known claustrophobia, and weight more than 200 lbs.

C. Measurements.

1. Menstrual and reproductive characteristics. Information on demographic and risk factors for breast cancer was obtained by questionnaire. This included age at the onset of menses, and details of prior exposure to oral contraceptives.

2. Anthropometric measures. A research assistant, trained by an instructor from the Department of Physical Education, University of Toronto, measured height, weight and waist and hip circumferences. Skinfold thickness was measured using calipers at subscapular, triceps and supra-iliac sites. Percent body fat was calculated using methods validated in adolescent girls [9,10], that use skinfold thickness measurements at triceps (T) and subscapular sites (S).

$$Percent\ body\ fat = \begin{cases} 1.33 \times (T+S) - 0.013 \times (T+S)^2 - 2.5 & if\ (T+S) \leq 35 \\ 0.546 \times (T+S) + 0.97 & if\ (T+S) > 35 \end{cases}$$

3. Diet. Dietary intake was assessed using a food frequency questionnaire adapted from the US DHQ II to optimise the capture of foods consumed by Canadians[22].

4.Magnetic resonance (MR) measures of breast water and fat. The first 181 young women included in the analysis were examined using a 1.5T Signa Cvi MR system (GE, Waukesha WI)[8]. The remaining 776 subjects were examined in a 3.0T scanner (Phillips). All scans were carried out in the prone position with commercially available breast coils from the respective vendors.

To determine if the data from these two scanners could be pooled we scanned 12 healthy volunteers on both scanners on the same day. Total fat and water measures from the two scanners were strongly correlated (R^2>0.99) but showed some underestimation of fat in the 3.0T protocol relative to the 1.5T protocol. A correction applied to the T3.0 protocol decreased the discrepancy [11].

With both scanners the sequence was calibrated using a series of home-built phantoms. Bi-monthly scans of three phantoms with known water/oil concentrations and various volumes using the same MR imaging protocol confirmed volume accuracy within 2% and water/oil content accuracy within 3%.

The output of the MR examination was a series of “slices” at 7-mm intervals through both breasts. The breast was distinguished from surrounding tissues on each slice by an observer using a semi-automated image analysis program, and the water and fat within each slice calculated and summed over all slices which acquires the water and fat signals with phase shifts of (0, pi, 2pi). The results shown are measurements in the right breast only and are expressed as percent water, total breast water and total fat. All measurements have been shown to be bilaterally symmetrical [12]. A small amount of water is also present in fat and allowance is made for this in calculating the water content of the breast used here.

5. Blood samples for measurement of glucose and insulin. Fasting blood samples were collected in the early morning after a 12-hour fast on the day of the MR examination and within 10 days of the first day of the most recent menstrual period. Serum was separated within 2 hours of collection and stored in 2ml aliquots at -70C until analysis.

6. Assays ofglucose and insulin. All assays in subjects from Phases 1 and 2 of recruitment were carried out in fasting serum at the same time in the Immunochemical Core Lab (ICL), Mayo Clinic Research Core Labs. The assays used, their inter- and intra- assay coefficients of variation, and lower limits of detection are shown in Supplementary material.

7. Calculation of insulin measures. We used fasting serum levels of glucose and insulin for each subject to calculate measures of insulin production, sensitivity and resistance using on-line software: https://www.dtu.ox.ac.uk/homacalculator/download.php version

2.2.3 for the HOMA model, that calculates[13] measures of insulin sensitivity (HOMA2-S), beta-cell function (HOMA2-B), and insulin resistance (HOMA2-IR).

D. Statistical methods. Nine hundred and fifty-seven young women were recruited and had breast MR and complete anthropometric measures. One hundred and sixty-eight subjects did not provide a blood sample. An additional five subjects whose fasting insulin or glucose values fell outside the limits set by the HOMA software, were excluded from the analysis of blood samples. Eighteen subjects had values for insulin or glucose that were outliers suggesting they were not fasting samples, leaving 766 subjects, of whom 25 did not complete a food frequency questionnaire. The remaining 741 subjects were included in the analysis. Details of these exclusions are given in the Supplementary Figure.

Means and standard deviations were calculated for selected characteristics. Total water and total fat by MR were log transformed for analysis.

The associations of MR breast tissue characteristics with dietary intakes and insulin were examined using univariable and multivariable regression models. The multivariable models included age, height and weight, percent body fat and waist-to-hip ratio at the time of the MR examination.

To illustrate graphically the main findings of regression analysis, we ran multivariable regression analyses with selected variables divided into quintiles. The statistical significance of the differences in breast measures of percent water, total water and total fat associated with increasing levels of these variables were assessed using tests for linear trend.

In mediation analysis, we examine the effect of an exposure on an outcome, which may be mediated by an intermediate variable. The exposure influences the intermediate variable, which in turn, affects the outcome[14]. The intermediate variable is generally referred to as the 'mediator' and the set of rules to quantify such effects is known as 'mediation analysis' [14,15] proposed a set of techniques to quantify the effect of an exposure on an outcome which is mediated by a mediator, namely the indirect effect, based on the assumption that there is no interaction between the exposure and the mediator. In the present context, we found that the interaction terms were non-significant and thus, we followed this approach. The details are described elsewhere (Baron and Kenny, 1986). This approach has been extended by several authors to allow for interaction between the exposure and the mediator [16,17].

The analyses were conducted using statistical software R version 2.15.1. All p-values were calculated from two-tailed tests of statistical significance. Statistical significance was declared at the 5% level. All p-values are adjusted for the variables shown in the Table footnotes.

Results

A. Characteristics of subjects. Table 1 shows selected characteristics of subjects enrolled in Phases 1 and 2 of the study. Subjects were in general similar in age, height and weight, and waist-to-hip ratio, but there was a statistically significant difference between phases in percent body fat. Energy intake, total protein, glycemic load, total carbohydrate intake and sucrose and fasting serum insulin also differed significantly between Phases one and two.

B. Association of nutrients with breast tissue composition. Table 2 shows the associations of selected nutrients and breast tissue measures of percent density, total breast water and total breast fat. The associations of nutrients and breast measures were examined using linear regression adjusted for age, height, weight, waist to hip ratio, percent body fat and food energy. Table 2 shows regression coefficients and associated p values.

Table 1
Selected characteristics of subjects overall and by phase of recruitment (mean (standard deviation)).

Risk Factors	Phase one ($n = 189$)	Phase two ($n = 577$)	Total ($n = 766$)	p-value
Height (cm)	165.69 (5.98)[3]	165.74 (6.23)[5]	165.73 (6.17)[1]	0.926
Weight (kg)	59.11 (9.34)[3]	58.58 (9.94)[5]	58.71 (9.79)[1]	0.518
Age at MRI (years)	16.59 (1.01)[3]	16.52 (0.85)[5]	16.53 (0.89)[1]	0.351
Waist-hip Ratio	0.72 (0.04)[3]	0.72 (0.04)[5]	0.72 (0.04)[1]	0.190
Percent of Body Fat	28.39 (6.16)[3]	26.25 (5.34)[5]	26.78 (5.63)[1]	<0.001
MRI Measurements				
Percent water (%)	51.81 (13.71)[3]	51.60 (13.61)[5]	51.65 (13.62)[1]	0.854
Total water (cm^3)	278.19 (135.31)[3]	299.36 (128.77)[5]	294.14 (130.64)[1]	0.053
Total fat (cm^3)	286.58 (206.71)[3]	308.89 (203.04)[5]	303.38 (204.04)[1]	0.192
Food Energy				
Total Energy (kcal)	1907.38 (815.40)[4]	1729.92 (733.94)[6]	1774.23 (758.44)[2]	0.006
Total Fat (g)	64.14 (32.94)[4]	61.54 (29.11)[6]	62.19 (30.11)[2]	0.311
Total Carbohydrate (g)	259.69 (113.26)[4]	234.66 (109.68)[6]	240.91 (111.04)[2]	0.008
Total Protein (g)	72.33 (31.95)[4]	66.88 (30.03)[6]	68.24 (30.59)[2]	0.035
Total Fibre	19.30 (8.22)[4]	19.97 (10.64)[6]	19.80 (10.09)[2]	0.434
Dietary Fibre (soluble) (g)	6.54 (2.85)[4]	6.70 (3.57)[6]	6.66 (3.40)[2]	0.580
Dietary Fibre (insoluble) (g)	12.62 (5.47)[4]	13.13 (7.16)[6]	13.00 (6.78)[2]	0.372
Glycemic Load	125.43 (58.83)[4]	111.08 (52.97)[6]	114.66 (54.81)[2]	0.002
Sucrose	48.29 (27.02)[4]	43.16 (26.38)[6]	44.44 (26.62)[2]	0.023
Hormones				
Insulin (μIU/mL)	11.20 (4.27)[3]	10.30 (4.09)[5]	10.52 (4.15)[1]	0.010
Growth Hormone (ng/mL)	1.26 (2.53)[3]	1.24 (2.59)[5]	1.24 (2.58)[1]	0.921
HOMA2 B	162.11 (43.19)[3]	161.10 (44.02)[5]	161.35 (43.79)[1]	0.784
HOMA2 S	84.49 (38.65)[3]	91.94 (38.08)[5]	90.10 (38.33)[1]	0.020
HOMA2 IR	1.39 (0.53)[3]	1.27 (0.50)[5]	1.30 (0.51)[1]	0.006

[1] $N = 766$, [2] $N = 741$, [3] $N = 189$, [4] $N = 185$, [5] $N = 577$, [6] $N = 556$.

Table 2
Association of selected nutrients with breast tissue composition (N = 741).

Outcome	Total energy	Total fat	Total Carbohydrate	Total Protein	Total fibre	Dietary Fibre (soluble)	Dietary Fibre (insoluble)	Glycemic load	Sucrose
Breast characteristics									
Percent water	0.0008 (0.12)	-0.005 (0.85)	-0.002 (0.86)	0.0214 (0.41)	**0.10 (0.049)**	0.25 (0.12)	**0.16 (0.04)**	-0.005 (0.74)	-0.008 (0.75)
log(Total water)	-0.00001 (0.55)	0.002 (0.11)	-0.0003 (0.39)	< 0.00001 (0.97)	0.003 (0.21)	0.006 (0.33)	0.004 (0.17)	-0.0008 (0.27)	-0.0006 (0.58)
log(Total fat)	**-0.00005 (0.03)**	0.002 (0.16)	-0.0002 (0.50)	-0.0009 (0.41)	-0.002 (0.48)	-0.004 (0.56)	-0.002 (0.47)	-0.0005 (0.44)	-0.0002 (0.85)

* Regression coefficients (p-values) are shown and are adjusted for age at MRI, height, weight, food energy, waist-hip ratio and percent of body fat

Insoluble and total dietary fibre were both significantly and positively associated with percent water. Insoluble and total dietary fibre were associated positively with total breast water and inversely with total breast fat, but neither association was statistically significant.

The direction and magnitude of these associations are illustrated in Fig. 1A and B that show the associations of the breast measure of percent water according to quintiles of insoluble and total and dietary fiber. Compared to the lowest quintile of fiber intake, percent water was 2.12% greater in the highest quintile of intake of insoluble fiber (P_{trend}=0.065) and 2.94% greater for the highest quintile of intake of total fiber (P_{trend}=0.002).

C. Associations of nutrients with insulin and HOMA measures. Table 3 shows the associations of the nutrients shown in Table 2 with fasting serum insulin, and the HOMA measures calculated from fasting serum insulin and fasting serum glucose. Soluble and insoluble fibres, and total fibre, were each significantly associated with fasting insulin and all of the HOMA measures. The associations of total and soluble fibre with fasting insulin, HOMA 2B and HOMA 2 IR were all inverse, and those with the HOMA2-S estimate of insulin sensitivity were positive. These nutrients explained between 9 and 11% of the variance in fasting insulin.

The direction and magnitude of these associations are illustrated in Fig. 1C -F that show the associations of log insulin and log HOMA 2-S according to quintiles of insoluble and total and dietary fiber. Compared to the lowest quintile of insoluble fiber intake, log insulin was 0.16 units greater in the highest quintile of intake of insoluble fiber (p_{trend}= 0.0.0001) and 016 units greater in the highest quintile of intake of total fiber ($_{Ptrend}$=0.002). Compared to the lowest quintile of fiber intake, the estimate of insulin sensitivity, log HOMA 2-S, was 0.22 units% greater in the highest quintile of intake of insoluble fiber (p_{trend}=0 < 0.0001) and 0.10 units greater for the highest quintile of intake of total fiber (p_{trend}=0.03).

D. Associations of nutrients with insulin and HOMA measures. Table 3 shows the associations of the nutrients shown in Table 2 with fasting serum insulin, and the HOMA measures calculated from fasting serum insulin and fasting serum glucose. Soluble and insoluble fibres, and total fibre, were each significantly associated with fasting insulin and all of the HOMA measures. The associations of total and soluble fibre with fasting insulin, HOMA 2B and HOMA 2 IR were all inverse, and those with the HOMA2-S estimate of insulin sensitivity were positive. These nutrients explained between 9 and 11% of the variance in fasting insulin.

The direction and magnitude of these associations are illustrated in Fig. 2 (C to F) that show the associations of log insulin and log HOMA 2-S according to quintiles of insoluble and total dietary fiber. Compared to the lowest quintile of fiber intake, insulin was 2.12% greater in the highest quintile of intake of insoluble fiber and 2.94% greater for the highest quintile of intake of total fiber. Compared to the lowest quintile of fiber intake, log HOMA 2-S was 2.12% greater in the highest quintile of intake of insoluble fiber and 2.94% greater for the highest quintile of intake of total fiber.

E. Associations of breast measures with fasting insulin and HOMA measures.

Table 4 shows the regression coefficients and associated p-values for the associations of percent water with fasting insulin and HOMA measures. The associations of percent water and total breast fat with fasting insulin and all HOMA measures were statistically significant after adjustment for age and all anthropometric measures. Insulin, glucose and the HOMA measures were not significantly associated with total breast water.

Table 3: Association between selected nutrients and hormones (N = 741).

Outcome	Total energy	Total fat	Total carbohydrate	Total protein	Total fibre	Dietary fibre (soluble)	Dietary fibre (insoluble)	Glycemic load	Sucrose
log(Insulin)	0.000003 (0.85)	0.001 (0.24)	-0.0003 (0.37)	-0.0005 (0.62)	**-0.007 (0.0004)**	**-0.01 (0.02)**	**-0.01 (0.0001)**	0.0003 (0.58)	-0.001 (0.14)
log(HOMA2B)	-0.00001 (0.43)	0.0003 (0.65)	<0.0001 (0.90)	-0.0001 (0.86)	**-0.003 (0.007)**	**-0.008 (0.049)**	**-0.005 (0.004)**	0.0004 (0.29)	-0.0008 (0.21)
log(HOMA2S)	-0.000005 (0.79)	-0.001 (0.22)	0.0003 (0.35)	0.0005 (0.61)	**0.007 (0.0004)**	**0.01 (0.02)**	**0.01 (0.0001)**	-0.0003 (0.62)	0.001 (0.14)
log(HOMA2IR)	0.000005 (0.79)	0.001 (0.22)	-0.0003 (0.35)	-0.0005 (0.61)	**-0.007 (0.0004)**	**-0.01 (0.02)**	**-0.01 (0.0001)**	0.0003 (0.62)	-0.001 (0.14)
log(Growth hormone)	0.00003 (0.71)	-0.0003 (0.94)	0.0007 (0.58)	-0.003 (0.44)	0.004 (0.61)	0.008 (0.74)	0.006 (0.57)	0.002 (0.45)	0.003 (0.50)
IGF-1[1]	0.0006 (0.91)	0.22 (0.46)	-0.03 (0.77)	0.37 (0.18)	-0.19 (0.73)	-0.69 (0.68)	-0.24 (0.77)	0.08 (0.66)	-0.49 (0.06)
IGFBP-3	0.00003 (0.55)	-0.005 (0.08)	0.0001 (0.88)	-0.0002 (0.95)	0.007 (0.16)	0.02 (0.24)	0.01 (0.13)	0.0004 (0.82)	-0.004 (0.10)

[1]N=711

* Regression coefficients (p-value) are presented

* Adjusted for age at MRI, height, weight, food energy, waist-hip ratio and percent body fat

Table 4
Association between hormones and breast tissue composition (n = 766).

Outcome	log(Insulin)	log(Glucose)	log(HOMA2S)	log(HOMA2B)	log(HOMA2IR)
Percent Water	**-3.52**	0.009	**3.38**	**-5.14**	**-3.38**
	(0.0005)	(0.87)	**(0.0008)**	**(0.0004)**	**(0.0008)**
log(Total water)	0.015	0.002	-0.02	-0.02	0.02
	(0.72)	(0.36)	(0.67)	(0.69)	(0.67)
log(Total fat)	**0.17**	0.002	**-0.16**	**0.20**	**0.16**
	(0.0001)	(0.46)	**(0.0001)**	**(0.001)**	**(0.0001)**
* Regression coefficients (p-value) are presented					
* Adjusted for weight, height, age at MRI, food energy, waist-hip ratio and percent body fat					

Figure 1G-H show the direction and magnitude of the association of log insulin and log HOMA2-S with percent water. Compared to the lowest quintile of log insulin, percent water was 4.23% lower in the highest quintile of log insulin (p_{trend}=0.001)). For log HOMA2-S percent water was 4.4% greater for the highest quintile of log HOMA2-S compared to the lowest quintile (p_{trend}=0.001).

D. Associations of breast measures with fasting insulin and HOMA measures.

Table 4 shows the regression coefficients and associated p-values for the associations of measures of breast tissue composition with fasting insulin and HOMA measures. The associations of percent water and total breast fat with fasting insulin and all HOMA measures were statistically significant after adjustment for age and all anthropometric measures. Insulin, glucose and the HOMA measures were not significantly associated with total breast water.

Figure 2 (G and H) show the direction and magnitude of the association of log insulin and log HOMA2-S with percent water. Compared to the lowest quintile of log insulin, log percent water was 4.23% lower in the highest quintile of log insulin. For log HOMA2-S percent water was 4.4% greater for the highest quintile of log HOMA2-S compared to the lowest quintile.

F. Mediation analysis: Associations of percent breast water with dietary fiber before and after adjustment for insulin and HOMA measures. Table 5 shows the regression coefficients and associated p-values for the associations of percent water with insoluble and total dietary fiber, before and after adjustment for fasting insulin and HOMA measures.

Table 5
Mediation analysis: Regression coefficients (p-values) showing the association between Percent water and nutrients before and after adjusting for hormones (N = 741).

		Exposure	
Potential Mediating Variables	**Adjustment**	**Dietary Fibre (insoluble)**	**Total Fibre**
log(Insulin)	(A) Model without potential mediator	**0.16 (0.04)**	**0.10 (0.049)**
	(B) Model with potential mediator	0.12 (0.11)	0.08 (0.12)
	(C) ACME	0.04 (95% CI: 0.01, 0.07)	0.02 (95% CI: 0.007, 0.046)
log(HOMA2B)	(A) Model without potential mediator	**0.16 (0.04)**	**0.10 (0.049)**
	(B) Model with potential mediator	0.13 (0.08)	0.08 (0.1)
	(C) ACME	0.03 (95% CI: 0.007, 0.05)	0.02 (95% CI: 0.003, 0.04)
log(HOMA2S)	(A) Model without potential mediator	**0.16 (0.04)**	**0.10 (0.049)**
	(B) Model with potential mediator	0.12 (0.10)	0.08 (0.12)
	(C) ACME	0.034 (95% CI: 0.01, 0.07)	0.02 (95% CI: 0.006, 0.04)
log(HOMA2IR)	(A) Model without potential mediator	**0.16 (0.04)**	**0.10 (0.049)**
	(B) Model with potential mediator	0.12 (0.10)	0.08 (0.12)
	(C) ACME	0.034 (95% CI: 0.01, 0.07)	0.02 (95% CI: 0.006, 0.04)

In mediation analysis, we estimate the average causal mediation effect (ACME), the average direct effect (ADE) and total effect. Total effect and ADE correspond respectively to the regression coefficients of fiber measures before and after adjusting for hormone measures. In principle, ACME is the effect of the fiber measures on percent water that is left out after we subtract the ADE from the total effect. In particular, for dietary fiber (insoluble), insulin and percent water, we can interpret ACME as, for every unit increase in dietary fiber (insoluble), we will observe 0.04 (0.01, 0.07) unit decrease in percent water on average, after we remove the association between dietary fiber (insoluble) and insulin, while keeping the other variables constant.

Similarly, for total fiber, insulin and percent water, we can interpret ACME as, for every unit increase in total fiber, we will observe 0.02 (0.007, 0.046) unit decrease in percent water on average after we factor out the association between total fibre and insulin, while keeping the other variables constant.

ACME is statistically significant for both insoluble and total dietary fiber measures, and we can conclude that there is evidence of insulin being a mediator for the association of both fibers with percent breast water [23–26].

Discussion

The present findings show that after adjustment for total energy intake and all anthropometric variables, insoluble and total dietary fiber were positively associated at age 15–18 with percent breast water. Insoluble and total dietary fibers were also associated inversely with fasting serum insulin and positively with the calculated HOMA2 S estimate of insulin sensitivity. Insulin was associated inversely with percent water and positively with total breast fat, and insulin sensitivity was associated positively with percent water and inversely with total beast fat. Mediation analysis suggests that the associations of dietary fiber with breast tissue composition may be mediated, at least in part, through the observed association with insulin or insulin sensitivity.

A limitation of the mediation analysis is that the cross-sectional design of the present study does not allow us to examine the direction of the associations shown between diet, insulin and breast tissue composition. However it is known from a randomized trial that dietary fiber influences insulin levels [14], and it is unlikely that breast tissue composition influences dietary intakes.

A Mendelian randomization analysis (by the Breast Cancer Association Consortium) in 182,306 participants of European ancestry, used genetic instruments for fasting insulin, fasting glucose, 2-h glucose, body mass index (BMI) and BMI adjusted waist-to-hip ratio (WHR). Confirmed the previously reported inverse association of genetically predicted BMI and WHR with risk of breast cancer and a positive association of predicted fasting insulin with risk of breast cancer. Mendelian randomization studies eliminate the potential reverse causation, and are less susceptible to bias and confounding than conventional observational studies.

Previous work in 957 subjects, that included the subjects in the present study, showed that PBW was associated with height (positively), and with weight, percent body fat and waist to hip ratio (all inversely). These variables explained 45% of the variance in PBW, and 53% of the variance in total breast fat, but only 5% of variance in total breast water. After adjustment for these anthropometric variables, fasting serum insulin (inversely), and insulin sensitivity (positively) were each significantly associated with PBW and, respectively, positively and inversely associated with total breast fat [27]. Further, administered insulin has been shown to be associated with greater percent density and greater dense volume in mammograms in adult women [28]. Existing knowledge thus suggests that the observed associations between dietary fibre, fasting serum insulin and breast tissue composition are plausible and may be causal.

As previously noted PBW is strongly correlated with mammographic density [30], a strong risk factor for breast cancer in adult women [29]. Previous studies have also shown that breast water is principally associated with the breast fibro-glandular tissue that is observed as radiologically dense [31]. Dietary fibre intake in young adults has been shown to be associated inversely with risk of breast cancer, and suggest that intake during adolescence may be particularly important [32].

Most previous studies of diet in childhood and mammographic density have used recall at ages 45–60 of diet in childhood, and mammograms obtained at ages 45–60. These studies have variously shown associations of red meat [15] and animal fat intake [16] with MD, no association of MD with dietary fiber[17], and no dietary associations with MD [18]. Jung et al used 3 X 24 hour recalls of diet collected from 182 girls at ages 10–19 years [19]. An association was found between mammographic density measured as percent dense breast volume determined by MR at ages 25–29, and intakes of sucrose and total carbohydrate at ages 10–19 years. Fiber was not associated with percent dense breast volume.

To our knowledge the present study is the largest to date of the nutritional associations with breast tissue composition in young women. The timing of the study in early life allows us to examine the association of breast tissue characteristics, measured using quantitative, volumetric methods, with contemporaneous measures of diet and body size, when the mean levels of percent breast water, and fasting serum levels of insulin, are greater than in adult life, and have not yet been influenced by parity, menopause, and the effects of ageing [20,21]. Dietary intakes were assessed using a food frequency questionnaire that has been modified by the addition of food group equivalents from the Canadian Diet History Questionnaire [22].

The present results require replication, but they suggest that interventions, either dietary or pharmacologic, directed at reducing insulin levels or sensitivity during childhood and adolescence, may be associated with a reduction of radiologically dense breast tissue in later life. It remains to be determined whether such a change in breast tissue composition in early life will be associated with a reduced risk of breast cancer in later life.

Conclusions

These results show an association of dietary fiber with breast tissue measures and suggest this association may be mediated, at least in part, by insulin or insulin sensitivity. These findings suggest a potential approach to breast cancer prevention in early life.

Declarations

Ethics approval and consent to participate: Ethics approvals were obtained from the University Health Network, Sunnybrook Hospital, and Women's College Hospital (all in Toronto) and from the Toronto District School Board, the Toronto Catholic District School Board, the York Region District School Board and the York Catholic District School Board.

Consent for publication; All authors have read and approved the paper.

Availability of data and materials: Data will be made available on request after ethics approval.

Competing interests. None

Funding: This research was supported by the Canadian Breast Cancer Research Alliance and the National Institutes of Health (RO1 CA135101-01A2). Funding for this work was also provided by Princess Margaret Cancer Centre, The Princess Margaret Cancer Foundation, and the Ontario Ministry of Health.

Authors contributions.

LM: Nutrient analysis, study supervision and writing.

LL: Study co-ordination, recruitment, data collection, and writing.

MT: Recruitment, data collection and writing.

SS: Statistical analysis

JZ: Statistical analysis

SC: MR breast examinations

GS: MR breast examinations

SM: Statistical analysis

NFB: Principal investigator, conception and design of study, obtained funding, overall supervision and writing.

1 Princess Margaret Comprehensive Cancer Centre, [2]Imaging Research, Sunnybrook Hospital, all Toronto, ON, Canada

All authors read and approved the submitted manuscript.

Acknowledgements: We thank the young women, their mothers, and the Toronto schools that participated in this research, and Drs. Sheila Dunn, Vivien Brown, Jennifer Rosset and Marla Shapiro for their assistance with the recruitment of subjects from family practices.

References

1. Ahlgren M, Melbye M, Wohlfahrt J, Sorensen TI. Growth patterns and the risk of breast cancer in women. The N Engl J Med 2004;351:1619-26.
2. Terry MB. Consistency, now what? Breast Cancer Res 2017;19:85.
3. McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: A meta-analysis. Cancer Epidemiology, Biomarkers & Prevention. 2006; 15:1159-69.
4. Boyd NF, Guo H, Martin LJ, et al. Mammographic density and the risk and detection of breast cancer. N Engl J Med 2007;356:227-36.

5. Boyd NF, Lockwood GA, Byng JW, Little LE, Yaffe MJ, Tritchler DL. The relationship of anthropometric measures to radiological features of the breast in premenopausal women. Br J Cancer 1998;78:1233-8.
6. Poon CS, Bronskill MJ, Henkelman M, Boyd NF. Quantitative magnetic resonance imaging parameters and their relationship to mammographic pattern. J Natl Cancer Inst. 1992;84:777-80.
7. Graham SJ, Bronskill MJ, Byng JW, Yaffe MJ, Boyd NF. Quantitative correlation of breast tissue parameters using magnetic resonance and X-ray mammography. Br J Cancer 1996;73:162-8.
8. Boyd NF, Martin LJ, Chavez S, et al. Breast-tissue composition and other risk factors for breast cancer in young women: a cross-sectional study. Lancet Oncology 2009;10:569-80.
9. Slaughter MH, Lohman TG, Boileau RA, et al. Skinfold equations for estimation of body fatness in children and youth. Hum Biol 1988;60:709-23.
10. Wong WW, Stuff JE, Butte NF, Smith EO, Ellis KJ. Estimating body fat in African American and white adolescent girls: a comparison of skinfold-thickness equations with a 4-compartment criterion model. Am J Clin Nutr 2000;72:348-54.
11. Csizmadi I, Kahle L, Ullman R, et al. Adaptation and evaluation of the National Cancer Institute's Diet History Questionnaire and nutrient database for Canadian populations. Public Health Nutr 2007;10:88-96.
12. Chavez S, Stanisz G. Comparing average breast fat content results from two different protocols at 1.5T and 3T: can the data be pooled? J Magn Reson Imaging 2014;40:890-8.
13. Hennessey S, Huszti E, Gunasekura A, et al. Bilateral symmetry of breast tissue composition by magnetic resonance in young women and adults. Cancer Causes Control 2014;25:491-7.
14. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care 2004;27:1487-95.
15. VanderWeele, T. (2015). Explanation in causal inference: methods for mediation and interaction. Oxford University Press.
16. VanderWeele, T. J. (2009). Marginal structural models for the estimation of direct and indirect effects. Epidemiology, pages 18–26.
17. Ghalandari H, Kamalpour M, Alimadadi A, Nasrollahzadeh J. Comparison of Two Calorie-Reduced Diets of Different Carbohydrate and Fiber Contents and a Simple Dietary Advice Aimed to Modify Carbohydrate Intake on Glycemic Control and Inflammatory Markers in Type 2 Diabetes: A Randomized Trial. Int J Endocrinol Metab 2018;16:e12089.
18. Shu X., Wu L., Khankari N.,et al Association of obesity and circulating insulin and glucose with breast cancer risk: a Mendelian randomization analysis. International J of Epidemiology. 2018. 1-12.
19. Linton L, Taylor M, Saha S,Zhu J**,** Chavez S, Stanisz G,P, Martin L, Dunn S**,** Minkin S, Boyd N. Body size, breast tissue composition, and circulating hormones and growth factors, at age 15-18: a cross-sectional study. (submitted for publication).
20. Tseng M, Olufade TO, Evers KA, Byrne C. Adolescent lifestyle factors and adult breast density in U.S. Chinese immigrant women. Nutr Cancer 2011;63:342-9.
21. Borgquist S, Rosendahl AH, Czene K, Bhoo-Pathy N, Dorkhan M, Hall P, Brand JS. Long-term exposure to insulin and volumetric mammographic density: observational and genetic associations in the Karma study. Breast Cancer Res. 2018 Aug 9;20(1):93.
22. Poon CS, Bronskill MJ, Henkelman M, Boyd NF. Quantitative magnetic resonance imaging parameters and their relationship to mammographic pattern. J Natl Cancer Inst 1992; 84:777- 810
23. McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev. 2006; 15:1159-69.29.
24. Graham SJ, Bronskill MJ, Byng JW, Yaffe MJ, Boyd NF. Quantitative correlation of breast tissue parameters using magnetic resonance and X-ray mammography. Br J Cancer 1996; 73:162-8.
25. Farvid, MS, Eliassen HA, Cho E, Liao X, . Chen WY, and Willett WC, Dietary Fiber Intake in Young Adults and Breast Cancer Risk. Pediatrics. 2016 Mar; 137(3):
26. Bertrand KA, Burian RA, Eliassen AH, Willett WC, Tamimi RM. Adolescent intake of animal fat and red meat in relation to premenopausal mammographic density. Breast Cancer Res Treat 2016;155:385-93.
27. Yaghjyan L, Ghita GL, Rosner B, Farvid M, Bertrand KA, Tamimi RM. Adolescent fiber intake and mammographic breast density in premenopausal women. Breast Cancer Res 2016;18:85.
28. Sellers TA, Vachon CM, Pankratz VS, et al. Association of childhood and adolescent anthropometric factors, physical activity, and diet with adult mammographic breast density. Am J Epidemiol 2007;166:456-64.

29. Jung S, Goloubeva O, Hylton N, et al. Intake of dietary carbohydrates in early adulthood and adolescence and breast density among young women. Cancer Causes Control 2018;29:631-42.
30. 30.. Vachon CM, Kuni CC, Anderson K. Association of mammographically defined percent breast density with epidemiologic risk factors for breast cancer (United States). Cancer Causes & Control : CCC 2000;11:653-62.
31. Byrne C, Schairer C, Wolfe J, et al. Mammographic features and breast cancer risk: effects with time, age, and menopause status. J Natl Cancer Inst 1995;87:1622-9.

Figures

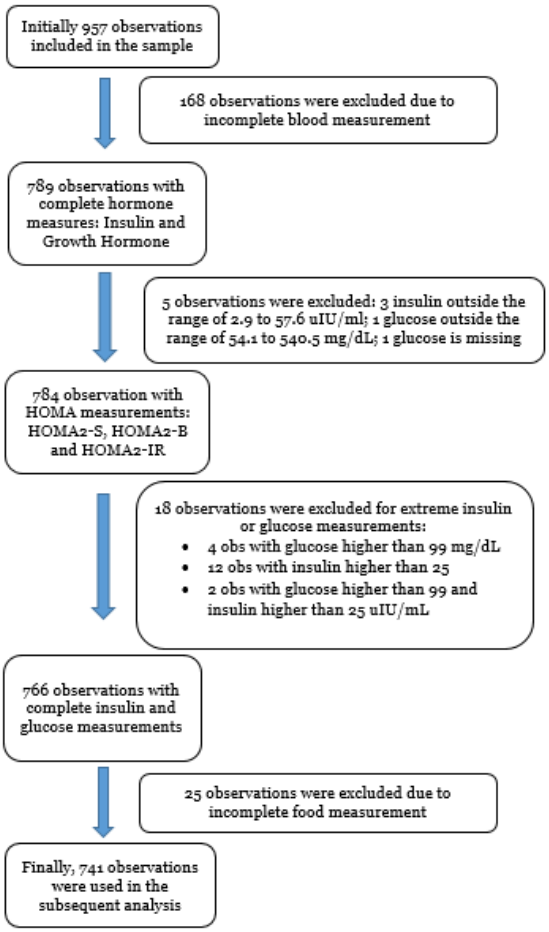

Figure 1

Data Exclusions: Subjects excluded from analysis.

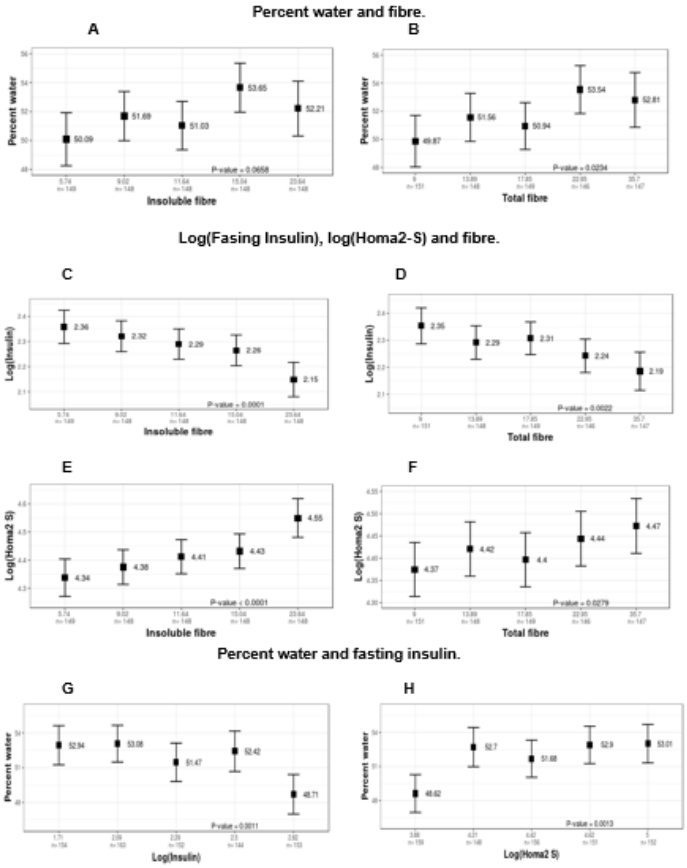

Figure 2

Associations between dietary fibre, percent breast water and fasting insulin. A: Association of percent breast water with insoluble fibre. B: Association of percent breast water with total fibre. C: Association of log insulin with insoluble fibre. D: Association of log insulin with total fibre. E: Association of log HOMA2-S with insoluble fibre. F: Association of log HOMA2-S with total fibre. G: Association of percent breast water with log insulin. H: Association of percent breast water with log HOMA2-S. All p values are from tests for trend, adjusted for height, weight, waist-hip ratio and percent body fat.