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Insulin resistance and incident gallbladder disease in pregnancy

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Abstract

Background and Aims—Insulin resistance is associated with prevalent gallstones, but its effect on initial gallstone formation is not well understood.

Methods—We conducted a nested case-control study to examine whether insulin resistance is a risk factor for initial gallbladder sludge and stone formation during pregnancy. Cases were 205 women with new gallbladder sludge and stones during pregnancy and the early post-partum. Controls were 443 randomly selected women without sludge or stones during pregnancy. Gallbladder ultrasounds were obtained in each trimester, and at 4–6 weeks postpartum. Fasting serum glucose, lipids, and insulin were measured at 26–28 weeks gestation. Insulin resistance was measured by the homeostasis model. Logistic regression was used to identify independent risk factors for gallstone formation.

Results—Insulin resistance was significantly greater in cases than controls on univariate analysis ($p < 0.001$). Pre-pregnancy body mass index was strongly associated with gallstone formation on univariate analysis ($p < 0.001$), but this association was diminished after adjusting for insulin resistance ($p = 0.01$). On multivariate analysis, insulin resistance was significantly associated with gallstone formation ($p = 0.004$), even after adjustment for pre-pregnancy body mass index and other confounding factors including HDL cholesterol and physical activity. This association was strongest in women with pre-pregnancy body mass index $< 30 \text{ kg/m}^2$.

Conclusions—Insulin resistance is a risk factor for incident gallbladder sludge and stones during pregnancy, even after adjustment for body mass index. Insulin resistance may represent a causal link between obesity and overweight and gallstones.

Introduction

In the United States, gallstone disease is one of the most common and costly of all digestive diseases, requiring more than 700,000 cholecystectomies annually¹. Prior cross-sectional studies have shown that body mass index (BMI), obesity, and diabetes are significantly associated with gallstones and biliary sludge^{2–6}. In addition, serum insulin and C-peptide levels are associated with gallstone prevalence². However, these studies examined prevalent

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gallstones, and therefore could not clearly define risk factors for initial development of gallbladder sludge or stones.

Parity is a significant risk factor for gallstones, and pregnancy is a high-risk period for gallstone formation^{7–9}. We have previously shown that the risk of forming gallbladder sludge or stones during pregnancy is significantly higher in obese women (BMI ≥ 30 kg/m²) than in normal weight (BMI < 25 kg/m²) or overweight (BMI 25.0–29.9 kg/m²) women¹⁰. Gallbladder sludge, a mixture of cholesterol and calcium bilirubinate crystals in bile, can be detected ultrasonographically and is generally felt to represent a precursor stage potentially leading to formation of actual gallstones¹¹. Insulin resistance, which increases with body mass index, is one possible mechanism linking obesity to gallstones^{2, 12–15}. However, not all studies confirm this association^{16, 17}. Previous studies examining insulin resistance and gallstones have been retrospective or cross-sectional, and it is difficult to determine if insulin resistance has a causal effect in gallstone formation. The aim of this study was to examine prospectively the effect of insulin resistance and gestational diabetes on initial development of gallstones during pregnancy.

Methods

We conducted a nested case-control study within a prospective cohort study of gallbladder disease during pregnancy¹⁰. The study was approved by the Institutional Review Boards of the University of Washington and Madigan Army Medical Center, and all women gave written informed consent. Potentially eligible women were approached for the study at their first obstetrics visit at Madigan Army Medical Center. Women were excluded because of age less than 18 years, language comprehension, plans to move away within 3 months, or presenting for prenatal care after 20 weeks gestation. Enrolled subjects underwent serial fasting gallbladder ultrasound examinations at 10–12 weeks gestation, 17–19 weeks gestation, 26–28 weeks gestation, and 4–6 weeks postpartum. Study questionnaires and interviews were completed at study entry, during the early third trimester, and in the early postpartum period, and covered past medical history, gastrointestinal symptoms, physical activity, behavioral factors such as smoking or alcohol intake, and dietary intake (entry and postpartum questionnaires only). Pre-pregnancy BMI was calculated from subjects' height measured at study entry and self-reported weight before pregnancy. Self-reported pre-pregnancy weight correlated significantly with weight measured at study entry (Spearman's correlation coefficient=0.94, $p<0.001$). Waist circumference was measured by research personnel at study entry (10–12 weeks gestation).

Fasting blood samples were drawn at 26–28 weeks gestation and tested for serum lipid and glucose levels. Gestational diabetes was diagnosed based on standard clinical criteria. Using serum samples stored at -70°C from this blood draw, total immunoreactive insulin was measured by a double-antibody radioimmunoassay developed by the Diabetes Endocrinology Research Laboratory at the University of Washington. We used the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) to assess insulin resistance from fasting glucose and insulin levels¹⁸. HOMA-IR is calculated as (fasting insulin [mU/ml] $\times$ fasting glucose [mmol/L]) $\div$ 22.5), with greater values of HOMA-IR indicating increasing degrees of insulin resistance. HOMA-IR has been shown to correlate well with insulin resistance in men and non-pregnant women as measured by more complicated measures such as the euglycemic hyperinsulinemic clamp¹⁸. Estradiol levels were measured by radioimmunoassay (ICN Biomedicals, Inc., Costa Mesa, CA) and progesterone levels by enzyme-linked immunoassay (Systems Labs, Inc., Webster, TX) from these samples.

Physical activity during the second and third trimesters was assessed by a modification of the CARDIA Physical Activity History instrument¹⁹. Women indicated the months in which they

participated in each of 8 categories of vigorous and 5 categories of moderate physical activity for at least 1 hour per week. Duration in hours and minutes per week was recorded. To account for a possible confounding effect of physical activity, we calculated the volume of physical activity in the second and third trimesters of pregnancy. We multiplied the average duration of activity per week of each activity by its metabolic equivalent units (METs) to determine the average MET-hours per week for each activity²⁰. One MET is the rate at which adults utilize energy at rest. We then summed over all activities to get an overall index of MET-hours. We divided women into quartiles of total activity based upon the calculated MET-hours for the second and third trimesters.

Gallbladder ultrasonography was performed with a standard imaging protocol using a 3.5–7.0 MHz rotatory sector scanning transducer (ATL Inc., Bothell, WA, or Acuson Corporation, Mountain View, CA). All study ultrasounds were performed by sonographers with special training in gallbladder ultrasound and with women fasting or having drank only sips of water. Findings were recorded by the sonographers, and images and findings were reviewed by one of two designated study radiologists with expertise in gallbladder ultrasound. Sludge was defined as the presence of low-level echoes that shift with position changes and without post-acoustic shadowing. Stones were defined as high-amplitude echoes greater than 2 millimeters in diameter with post-acoustic shadowing. In 10,887 scans performed in the entire study, there were discrepancies between the radiologist's and the sonographer's readings in 70 regarding the diagnosis of sludge ($\kappa=0.93$) and in 25 regarding the diagnosis of stones ($\kappa=0.98$). In case of discrepancy, the radiologist's reading was accepted as correct.

Of the 8,929 women approached, 4,897 (55%) were eligible and interested for the parent cohort study. We excluded an additional 208 women with stones on entry ultrasound and 33 women with a prior cholecystectomy. Two interpretable study ultrasounds were available for 3,254 women. For this case-control study, cases ($n=205$) were defined as women without gallbladder sludge or stones on the entry ultrasound, and who subsequently developed new gallbladder sludge or stones on any study ultrasound. Controls ($n=512$) were randomly selected using a random number table from subjects in the parent study without gallbladder sludge or stones on any ultrasound. Sixty-nine controls did not have serum available, leaving 443 controls for analysis. The timing and number of available ultrasounds was similar in cases and controls (3.1 ± 0.7 in cases vs. 3.2 ± 0.7 in controls, $p=0.47$). Cases and controls were not matched on any variables, and women with pre-existing or gestational diabetes were eligible for inclusion in this study.

The distributions of categorical and continuous variables were compared by the chi-square test, Student's t-test, or the Wilcoxon sign-rank test as appropriate. Multiple logistic regression was used to examine the association of insulin resistance with gallbladder disease after adjustment for other factors (Stata 8.0, Stata Corp., College Station, TX). Variables considered for inclusion were those previously shown to be associated with gallbladder disease (parity, weight gain during pregnancy, HDL cholesterol)¹⁰, demographic variables (age, race, ethnicity), alcohol consumption, and variables potentially related to maternal weight (pre-pregnancy body mass index, waist circumference at entry, waist-hip ratio at entry) or insulin resistance (glucose and lipid levels). Variables that were significant with $p<0.1$ were retained in the final model. Significance of the regression models was tested with the likelihood-ratio statistic.

Results

Cases and controls were similar in age, race, and parity (Table 1). Women who developed sludge or stones were more likely to be Hispanic than control women. In addition, women who developed sludge or stones had significantly higher pre-pregnancy BMI and waist circumference, but not waist-hip ratio, at entry. Both weight gain during pregnancy and serum

HDL cholesterol levels were inversely associated with the risk of developing sludge or stones. Fasting serum glucose levels and the prevalence of clinically diagnosed gestational diabetes mellitus were similar in cases and controls. However, serum insulin levels and HOMA-IR were significantly higher in cases than in controls, suggesting greater degrees of insulin resistance in women who formed sludge or stones even in the absence of clinically diagnosed gestational diabetes.

In men and non-pregnant women, insulin resistance is associated with overweight and obesity, and therefore weight status may confound the relationship between insulin resistance and gallstone formation. In our subjects, HOMA-IR increased with pre-pregnancy overweight or obesity, compared to normal weight status (Table 2). In univariate analyses stratified by pre-pregnancy weight status, HOMA-IR was significantly greater in normal weight or overweight cases, compared to controls of comparable weight status (Table 2). In obese women (BMI $\geq$ 30 kg/m²), HOMA-IR did not differ significantly between cases and controls.

Similarly, glucose and lipid levels are associated with insulin resistance, and therefore may confound the relationship between insulin resistance and gallbladder disease during pregnancy. Fasting glucose and triglycerides at 28 weeks gestation increased significantly, while LDL cholesterol levels decreased with increasing HOMA-IR (Table 3). There was a strong trend towards decreasing HDL cholesterol levels with increasing HOMA-IR, but no association of total cholesterol levels with HOMA-IR.

We developed logistic regression models to further characterize the relationship between gallstone formation, pre-pregnancy overweight or obesity, and insulin resistance (Table 4). We initially considered for inclusion all variables associated with case status with $p < 0.1$. We also included other variables considered to be potential confounders, such as waist-hip ratio and physical activity, measured as MET-hrs expended weekly in the second and third trimesters. We first developed models examining either pre-pregnancy weight status or insulin resistance, but not both. Women were categorized into quartiles for degree of insulin resistance (measured by HOMA-IR).

In separate models, insulin resistance (Table 4, model 1) and pre-pregnancy overweight or obesity (Table 4, model 2) remained independent risk factors for gallstone incidence after adjustment for the other variable, although the strength of the association was attenuated. In a final multivariate model examining both HOMA-IR and pre-pregnancy weight status, insulin resistance remained a strong risk factor for gallstone formation, even after adjustment for pre-pregnancy overweight or obesity (Table 4, model 3). In this model, pre-pregnancy with status was still significantly associated with gallstone development after adjustment for insulin resistance, but the strength of the association was attenuated. Similar results were seen when HOMA-IR or pre-pregnancy BMI were entered into the model as a continuous, rather than categorical, variables, or when estradiol and progesterone levels were added to these models (data not shown). We have previously shown that estradiol and progesterone levels at 26–28 weeks gestation were not associated with new gallbladder sludge or stones in our population¹⁰. In addition, Hispanic women may be genetically predisposed to cholesterol gallstone formation. Exclusion of such women from our analyses again did not significantly change these results (data not shown).

To further characterize the role of insulin resistance, we performed additional analyses stratified by pre-pregnancy weight status (Table 5). These analyses show that insulin resistance was a significant risk factor for gallstone development in normal weight or overweight women, but not in obese women. The interaction between pre-pregnancy weight status and insulin resistance in influencing risk for new gallbladder sludge or stones was of borderline significance ($p = 0.08$).

Discussion

We prospectively studied risk factors for incident gallbladder sludge and stones during pregnancy, and measured fasting serum glucose, lipids, and insulin during the period of gallstone formation. Our results are consistent with prior studies showing that fasting serum insulin, which may be used as a surrogate measure for insulin resistance, is a risk factor for prevalent gallstones^{2, 14, 21–23}. We found that women who formed gallbladder sludge or stones were significantly more insulin resistant, as measured with HOMA-IR. This association remained strong even after adjusting for possible confounding factors including pre-pregnancy BMI, lipid and glucose levels, waist-hip ratio, and physical activity during pregnancy. This association was strongest for women whose pre-pregnancy BMI was less than 30 kg/m².

Prior studies have examined insulin levels and insulin resistance in subjects with prevalent gallstones², but not during the initial period of gallstone formation. For the majority of women in this study, gallbladder sludge or stones were first noted on the early 3rd trimester or postpartum ultrasounds. We measured serum insulin, glucose, and lipids at 27–29 weeks gestation, at a time when gallbladder sludge and stones were initially forming or had recently formed. Our study therefore extends previous findings and suggests that insulin resistance may be a pathogenic factor for gallstone formation. The effect of pre-pregnancy BMI on gallstone formation was attenuated after adjusting for insulin resistance, suggesting that insulin resistance may be part of the causal pathway linking obesity and gallstones.

The exact mechanisms whereby insulin resistance leads to gallstone formation are not clear. Cholesterol is the primary constituent of gallstones formed during pregnancy. Cholesterol gallstone formation requires several pathogenic factors, including supersaturation of hepatic bile with cholesterol and altered gallbladder motility. Hyperinsulinemia and insulin resistance may affect either of these factors. Hyperinsulinemia has direct effects on hepatic lipid metabolism, increasing cholesterol synthesis via activity of the hydroxyl-methylglutaryl coenzyme A reductase enzyme²⁴ and increasing hepatic uptake of LDL cholesterol²⁵. Insulin resistance is also associated with lower serum levels of HDL cholesterol, a known risk factor for prevalent gallstones²⁶. In one study, administration of insulin in diabetics increased biliary cholesterol saturation²⁷. Some, but not all, studies have shown greater degrees of biliary cholesterol saturation in patients with type II diabetes compared with controls^{28, 29}.

Insulin inhibits basal and cholecystokinin-stimulated gallbladder motility, and gallbladder dysmotility has been documented in patients with clinically diagnosed type II diabetes^{30, 31}. In animal models, non-obese, diabetic mice have diminished gallbladder contractility and rapid formation of cholesterol crystals³², while gallbladder contractility correlates inversely with glucose and insulin levels in obese animals³³. Insulin resistance is associated with gallbladder dysmotility in non-obese, non-diabetic humans³⁴. Therefore, insulin resistance, even in the absence of obesity, may be lead to gallbladder sludge and stone formation, either by causing gallbladder dysmotility or by altering biliary lipid secretion.

Although our study strongly suggests that insulin resistance is a risk factor for gallstone formation, we cannot prove a direct mechanistic role from the analyses performed here. Insulin resistance may be a surrogate for other, undefined pathophysiologic mechanisms that lead to gallstone formation, rather than a direct underlying cause. We cannot account for all such potential pathophysiologic mechanisms in this analysis. For example, other, undefined abnormalities in lipid metabolism may lead to gallstone formation, and also lead independently to insulin resistance. We measured levels of insulin, glucose, estradiol, and progesterone at only one point in time, and levels earlier in pregnancy or changes in these levels may also be directly relevant. Therefore, our results should be viewed as hypothesis-generating, rather than

hypothesis-testing. Further studies in animal models are needed to help clarify this potential relationship between insulin resistance and gallstone formation.

In summary, we have shown that insulin resistance is strongly associated with gallstone formation during pregnancy, independent of pre-pregnancy BMI. This association was strongest in women who were normal weight or overweight, but was not present in obese women. The effect of body weight was attenuated after adjusting for insulin resistance, suggesting that insulin resistance may represent an intermediate step linking body weight to gallstone formation. Although this study was performed in pregnant women, these results may also explain the association between obesity and gallstone disease that is well-documented in non-pregnant women and, to a lesser degree, in men. Further studies in non-pregnant populations are needed to confirm this possibility, and studies in animal models are needed to clarify the underlying mechanisms of this association.

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Abbreviations

HOMA-IR	Homeostasis model of insulin resistance
BMI	Body mass index
MET	Metabolic equivalent unit

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Table 1

Selected characteristics of study subjects

	No sludge or stones (n=443)	Sludge or stones (n=205)	p-value
Age, years (mean $\pm$ SD)	25.1 $\pm$ 4.8	24.9 $\pm$ 4.4	0.64
Race (n, %)			0.20
White	324 (72.3)	148 (70.5)	
African-American	53 (11.8)	20 (9.5)	
Asian/Pacific Islander	26 (5.8)	10 (4.8)	
Native American	8 (1.8)	2 (1.0)	
Other/mixed	37 (8.3)	30 (14.2)	
Hispanic ethnicity (n, %)	39 (8.7)	34 (16.2)	0.004
Parity (median, interquartile range)	1 (0–2)	1 (0–2)	0.93
Waist circumference at entry, cm (mean $\pm$ SD)	86.2 $\pm$ 13.4	88.9 $\pm$ 14.7	0.02
Waist-hip ratio at entry (mean $\pm$ SD)	0.83 $\pm$ 0.07	0.83 $\pm$ 0.07	0.52
Weight gain during pregnancy, kg (mean $\pm$ SD)	14.2 $\pm$ 5.9	12.7 $\pm$ 7.3	0.007
Pre-pregnancy body mass index, kg/m ² (mean $\pm$ SD)	24.5 $\pm$ 5.0	26.9 $\pm$ 6.5	<0.001
Alcohol use during pregnancy (n, %)	130 (29.0)	48 (22.9)	0.09
MET-hours per week of vigorous physical activity, 2 nd trimester (median, interquartile range)	118 (62, 186)	95 (44, 171)	0.01
MET-hours per week of vigorous physical activity, 3 rd trimester (median, interquartiles range)	102 (50, 164)	99 (40, 172)	0.26
Gestational diabetes (n, %)	8 (1.8)	3 (1.4)	0.88
Total cholesterol, mg/dL (mean $\pm$ SD)	237.2 $\pm$ 41.0	237.1 $\pm$ 41.8	0.97
HDL cholesterol, mg/dL (mean $\pm$ SD)	66.1 $\pm$ 17.3	61.2 $\pm$ 15.9	<0.001
LDL cholesterol, mg/dL (mean $\pm$ SD)	130.5 $\pm$ 39.6	131.9 $\pm$ 39.0	0.68
Triglycerides, mg/dL (mean $\pm$ SD)	207.1 $\pm$ 83.0	217.0 $\pm$ 70.1	0.14
Glucose, mg/dL (mean $\pm$ SD)	74.2 $\pm$ 8.6	75.4 $\pm$ 7.2	0.09
Insulin, mU/L (median, interquartile range)	16.2 (11.1, 23.4)	20.1 (13.5, 28.1)	<0.001
HOMA-IR (median, interquartile range)	2.93 (1.91, 4.33)	3.69 (2.40, 5.48)	<0.001

Table 2

Insulin resistance and incident gallbladder disease, stratified by pre-pregnancy body mass index

HOMA-IR (median, interquartile range)				
Pre-pregnancy body mass index	All women (n=648)	No sludge or stones (n=443)	New sludge or stones (n=205)	p-value *
<25 kg/m ²	2.64 (1.77, 3.69)	2.56 (1.73, 3.60)	3.00 (2.04, 3.83)	0.06
25–29.9 kg/m ²	3.87 (2.56, 5.42)	3.49 (2.27, 5.03)	4.83 (3.46, 6.17)	0.001
≥30.0 kg/m ²	5.49 (3.78, 7.47)	5.56 (3.89, 7.80)	4.82 (3.54, 7.02)	0.30

* Comparison of women with new sludge/stones vs. women without sludge/stones

Table 3
Insulin resistance, glucose and lipid levels at 27–29 weeks gestation

	HOMA-IR (quartiles)				
	0.70–2.08	2.09–3.18	3.19–4.84	4.85–18.98	p-value for trend
Glucose, mg/dL *	68.8± 5.0	72.7± 5.2	74.8± 5.5	82.0± 9.7	<0.001
Triglyceride, mg/dL *	193.1± 83.1	190.9± 61.9	213.8± 79.4	243.3± 79.7	<0.0001
Total cholesterol, mg/dL *	235.1± 40.3	243.0± 38.4	234.7± 41.7	235.9± 44.1	0.64
HDL cholesterol, mg/dL *	66.1± 17.0	65.6± 15.3	62.5± 16.0	63.9± 19.3	0.06
LDL cholesterol, mg/dL *	130.4± 38.4	139.2± 35.7	129.9± 40.4	124.2± 41.8	0.02

* All values are mean ± SD.

Table 4

Multivariate models of insulin resistance, body mass index, and gallstone formation

	Unadjusted odds ratio (95%CI)	Model 1 [*] adjusted odds ratio (95% CI)	Model 2 [†] adjusted odds ratio (95% CI)	Model 3 [§] adjusted odds ratio (95% CI)
HOMA-IR, quartiles			N/A	
0.70–2.08	1.00	1.00		1.00
2.09–3.18	1.40 (0.83, 2.34)	1.30 (0.78, 2.21)		1.31 (0.77, 2.25)
3.19–4.84	2.88 (1.76, 4.71)	2.40 (1.44, 3.98)		2.48 (1.47, 4.18)
4.85–18.98	2.58 (1.58, 4.22)	1.77 (1.03, 3.05)		2.06 (0.17, 3.62)
p-value	<0.001	0.005		0.004
Pre-pregnancy body mass index, kg/m ²		N/A		
<25.0	1.00		1.00	1.00
25.0–29.9	1.30 (0.87, 1.95)		1.08 (0.70, 1.66)	1.12 (0.72, 1.75)
≥30.0	2.60 (1.66, 4.08)		2.01 (1.23, 3.28)	2.17 (1.29, 3.66)
p-value	<0.001		0.01	0.01

* Adjusted for pre-pregnancy overweight or obesity

† Adjusted for HOMA-IR

§ Includes both pre-pregnancy overweight/obesity and HOMA-IR. Also adjusted for Hispanic ethnicity, HDL cholesterol at 27–29 weeks, waist-hip ratio, and 2nd and 3rd trimester physical activity

Table 5

Insulin resistance and gallstone development, stratified by pre-pregnancy body mass index

HOMA-IR, quartiles	Odds ratio (95% confidence interval) *		
	BMI <25.0 kg/m ² (n=397)	BMI 25.0–29.9 kg/m ² (n=151)	BMI 30.0 kg/m ² (n=100)
0.70–2.08	1.00	1.00	1.00
2.09–3.18	1.21 (0.66, 2.24)	1.65 (0.33, 8.16)	1.74 (0.21, 14.32)
3.19–4.84	2.52 (1.34, 4.71)	4.17 (0.96, 18.21)	1.51 (0.22, 10.56)
4.85–18.98	1.51 (0.69, 3.31)	6.96 (1.59, 30.44)	1.10 (0.17, 7.00)
p-value	0.03	0.02	0.88

* Adjusted for Hispanic ethnicity, HDL cholesterol at 27–29 weeks, waist-hip ratio, and 2nd and 3rd trimester physical activity