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Birth control pills and risk of hypothyroidism based on National Health and Nutrition Examination Survey (NHANES) from 2007 to 2012

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Study conception and design: Yuxuan Qiu, Jingqiang Zhu and Anping Su; Acquisition of data: Yuxuan Qiu and Qingyu Fu. Analysis and interpretation of data: Yuxuan Qiu and Yuanyuan Hu; drafting of manuscript: Yuxuan Qiu, Yuanyuan Hu, Zhichao Xing. Critical revision of manuscript: Jingqiang Zhu and Anping Su.

Data sharing statement:

All data were collected from National Health and Nutrition Examination Survey. We declare the authenticity and transparency of the data. Extra data can be available from e-mailing to Yuxuan Qiu.

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Abstract

Objective: The association between use of birth control pills and thyroid functions in women have not ever been well studied, but potential risk has been implicated by small sample-sized studies.

Methods: We included female subjects aging >18 who met inclusion criteria US National Health and Nutrition Examination Survey, 2007 to 2012. History of taking birth control pills were based on responses in the reproductive health questionnaire. Participants not on antithyroid medication with a TSH > 5.6 mIU/L and those on thyroid hormone replacement regardless of TSH were categorized as hypothyroid. Participants not on thyroid hormone replacement or antithyroid medication who had a TSH between 0.34 and 5.6 mIU/L were categorized as euthyroid. Multivariate logistic regression analyses were performed to determine the association between use of birth control pills and hypothyroidism.

Results: A total of 5,116 female adults were included. Multivariate logistic regression analysis adjusted for covariables demonstrated a significant association between using birth control pills more than 10 years and hypothyroidism (OR, 3.837; 95% CI, 1.402 to 10.500; P=0.009).

Conclusions: Longer history of using birth control pills was strongly associated with hypothyroidism, especially more than 10 years.

Key words: birth control pills, contraception, hypothyroidism, NHANES

Abbreviations: BMI, body mass index; CI, confidence interval; FT4, free thyroxine; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; TSH, thyroid-stimulating hormone;

Article summary

Strengths and limitations of this study

This study found an association between oral contraception and hypothyroidism from a large amount of data National Health and Nutrition Examination Survey, which has been put forward in some polit studies. However, there might be some confounding factors in our data analysis though most of them have been adjusted.

Bullet points

- Hypothyroid status occurs more frequently in female population taking oral contraception.
- Longer history of oral contraception use might associate with hypothyroidism.
- Taking oral contraception for over 10 years independently raises risk of hypothyroidism.

Introduction

Birth control pills have developed quickly and been widely used by an increasing number of women of child-bearing potential since its introduction[1]. As the most common form of effective and reversible contraception, the prevalence of birth control pills in women aged 15 to 45 was 17% among women and 27.3% among all contraception methods in the USA. Moreover, use of birth control pills declined as age increase: 54% of contraceptors were under 20 years old, 35% at 20 to 40, and only 11% at age 40-45[2]. Youth and popularization of birth control pills warrants further investigation on safety, especially research into the long-term safety. Birth control pills were firstly designed for ovulation inhibition thus applying in birth control[3]. Over time, they not only helped avoiding unwanted pregnancies but also used in treatment for abnormal uterine bleeding, endometriosis, menstrual and hormonal disorder, etc. Additionally, long-term of taking birth control pills (≥10 years) could significantly decrease the risk of ovarian and endometrial cancer[4]. However, they could also bring many adverse effects including increase risk of hypertension, thromboembolic events, breast cancer, serious autoimmune diseases, and especially, endocrine related dysfunctions[5]

[6].

Thyroid hormone, one of the most notable endocrine hormones, gets involved in almost all nucleated cells and are crucial for normal growth, energy metabolism and reproduction. Hypothyroidism is the most frequent pathological hormone insufficiency whose prevalence was 4.6% in the USA according to the NHANES III study. It accounts approximately 3-7 times higher in women compared to men, and its incidence raises with age[7 8]. Several drugs could cause hypothyroidism and the most notable were lithium, amiodarone and tyrosine kinase inhibitors[8]. However, considering its higher incidence in women, there may be an association between medication commonly used by women and thyroid function. A literature review summarized one cohort and two case-control studies and reported the use of birth control pills was linked to a lower incidence of hyperthyroidism and potential relationship was found between use of birth control pills and hypothyroidism[5 9].

In other words, the relationship between use of birth control pills and hypothyroidism was unobserved and existed studies were limited by their sample size and follow-up duration. We examined the NHANES database in representative of the US population to figure out whether a correlation exists between use of birth control pills and hypothyroidism.

Materials and methods

Patient and Public Involvement

We conducted a retrospective analysis of a cohort in the US population the National Health and Nutrition Examination Survey (NHANES), a periodic survey performed by National Center for Health Statistics (NCHS) with an informed consent to every participant. Therefore, there was no need for any ethical consent in this study. NHANES includes extensive demographic data, physical examinations, laboratory tests, healthy related questionnaires and lists of prescription medications. Data of NHANES 2007 to 2012 is the only continuous collection providing information of reproductive health questionnaires and laboratory tests of thyroid function in US women. We included women who offered information of taking birth control pills in the reproductive health questionnaire, reported thyroid medication use and had thyroid function laboratory test value.

Definitions of thyroid condition

Thyroid condition was estimated via reported currently taking medications and thyroid-stimulating hormone (TSH) testing. NHANES documentation provides a reference range of 0.34 to 5.6 mIU/L for normal TSH based on manufacturer's guidelines. Participants were defined as hyperthyroid if they reported currently taking methimazole or propylthiouracil, regardless of TSH level, or if their TSH level was <0.34 mIU/L, based on 2016 the American Thyroid Association guidelines[10]. If the remaining participants reported currently taking levothyroxine regardless of TSH level, or if their TSH level was >5.6 mIU/L, they were defined as hypothyroid. Participants were defined as euthyroid if they were included in neither hyperthyroid nor hypothyroid.

Covariables and grouping

Demographic information on age, race/ethnicity and education was recorded at time of the interview. Body mass index (BMI) was coded into four categories based on standard cutoffs: underweight (<18.5 kg/m^2), normal BMI (18.5 to <25 kg/m^2), overweight (25 to <30 kg/m^2), and obese (≥30 kg/m^2). Smoking was coded as current, former, or never, and alcohol use was coded in four categories from never up to three or more drinks per day. Self-reported history and current knowledge of thyroid disease were included.

Participants were divided into two groups according to the reproductive health questionnaire

whether they have ever taken birth control pills. If taken, they were divided into history of taking birth pills group (history group); if never taken, they were divided into no history group. Reproductive variables such as first menstrual period, pregnancy history, menopause status, history of hormone use were covered.

Statistical analysis

Statistical analyses were performed in StataSE 14.2 (StataCorp LLC, College Station). Chi-square tests were used in descriptive tables on population characteristics; multivariate logistic regression was used to estimate the odds of a hypothyroid diagnosis among participants with history of taking birth control pills. Coefficients of logistic regression models presented include an unadjusted model, followed by model 1, adjusting for demographic covariables including age, race, education, model 2, adding self-related covariables including BMI, smoke status, alcohol use, self-reported history of thyroid disease, currently thyroid disease, and model 3, continuously adding gynecological covariables including first menstrual period, pregnancy history, menopause status, history of hormone use and all variables from model 2. History of taking birth control pills were subgroups into history less than 1 month, 1 month to 1 year, 1 to 2 years, 2 to 10 years and >10 years. Statistical significance was set at $P < 0.05$.

Results

Population characteristics

The total number of participants in the 2007 to 2012 NHANES was 30,442. Only 5116 female subjects met the inclusion criteria, including 2082 and 3034 women who never and ever taking birth control pills, respectively (Figure 1). Among the 3034 women who reported history of taking birth control pills, 210 (6.9%) have taken birth control pills less than 1 month, 864 (28.5%) have a history of 1 month and 1 year, 329 (10.8%) of 1 and 2 years, 1235 (40.7%) of 2 to 10 years, and 376 (12.4%) of longer than 10 years. Table 1 listed the demographics and health characteristics of the history group and no history group. Younger women (age < 65 years), non-Hispanic whites, participants with higher education, obese participants, currently smoking, higher alcohol consumption, history of pregnancy and later first menstrual period (age ≥ 13 years) had higher proportions of history group than their counterparts. Menopause status, age of last menstruation and use of hormone including estrogen and progestin (not including birth control pills) were not different between two groups. Among the 5116 participants, 830 were identified as hypothyroid, 4194 as euthyroid, and 92 as hyperthyroid. Participants in history group were more frequently developing a hypothyroid status with no difference in history and current knowledge of thyroid disease.

Association between history of taking birth control pills and hypothyroid

According to univariate analysis, any history of taking birth control pills carried an odds ratio (OR) of 1.280 with a 95% confidence interval (CI) of 1.104 to 1.484 for developing hypothyroidism (P=0.001). Participants with a history of 2 to 10 years (OR, 1.329; 95% CI, 1.108 to 1.595; P=0.002) and >10 years (OR, 1.865; 95% CI, 1.440 to 2.415; P=0.000) were more likely to have a hypothyroidism diagnosis. After adjusting for model 1 (demographic covariables including age, race, education), any history of taking birth control pills remained high risk of hypothyroidism (OR, 1.245; 95% CI, 1.043 to 1.486; P=0.015). Participants with a history of 1 month to 1 year (OR, 1.293; 95%, 1.021 to 1.636; P=0.033), 2 to 10 years (OR, 1.262; 95% CI, 1.022 to 1.559; P=0.030) and >10 years (OR, 1.555; 95% CI, 1.167 to 2.072; P=0.003) were of higher risk of developing a hypothyroid status. However, after adjusting for model 2, adding self-related covariables including BMI, smoke status, alcohol use, self-reported history of thyroid disease, currently thyroid disease,

women with a history of taking birth control pills for more than 10 years carried higher risk of hypothyroidism (OR, 4.025; 95% CI, 1.489 to 10.879; P=0.006). Similarly, after adjusting for model 3, adding gynecological covariables including first menstrual period, pregnancy history, menopause status, history of hormone use and all variables from model 2. women with a history of taking birth control pills for more than 10 years existed higher risk of hypothyroidism (OR, 3.837; 95% CI, 1.402 to 10.500; P=0.009). All details were displayed in Table 2 and Figure 2.

Discussion

To the best of our knowledge, this is the first study revealed a strong association between long time use of birth control pills and hypothyroidism. Based on a large number of participants in NHANES, the incidence of hyperthyroidism increased significantly along with the history of birth control pills use, even have adjusted. Participants with a history of 1 month to 1 year (OR, 1.293; 95%, 1.021 to 1.636; P=0.033), 2 to 10 years (OR, 1.262; 95% CI, 1.022 to 1.559; P=0.030) and >10 years (OR, 1.555; 95% CI, 1.167 to 2.072; P=0.003) were of higher risk of developing a hypothyroid status adjusting for demographic covariables including age, race, education. A history of taking birth control pills for more than 10 years carried significantly higher risk of hypothyroidism (OR, 3.837; 95% CI, 1.402 to 10.500; P=0.009) after adjusting for all considered variates including age, race, education, BMI, smoke status, alcohol use, self-reported history of thyroid disease, currently thyroid disease, first menstrual period, pregnancy history, menopause status, medical use of hormones.

Currently, there are quantity types of birth control pills available. Combination oral contraceptives containing both estrogen and progestin and progestin-only contraceptives are the two major types with many variations in the composition of the components[3]. In 1978, Frank et al published the results of a cohort study of 23,000 women currently taking contraceptive pills and a similar number of controls who never taken. It lasted for 14 months and indicated oral contraceptives exert a protective effect against thyroid myxoedema with a relative risk of 0.57. Vestergaard et al conducted a case-control study compromising 628 patients with autoimmune hypothyroidism and equal controls in a low-iodine intake area. It suggested ever use of oral contraceptives was associated with a slightly lower risk of Graves' disease in women, but not of autoimmune hypothyroidism[11]. Another case-control study conducted by Strieder et al held opposite opinion that neither ever use (OR, 4.20; 95% CI, 0.55 to 32.43) or current use (OR 0.89; 95% CI, 0.38 to 2.10) of oral contraception was associated with hypothyroidism[12]. Besides, 12 weeks of estrogen therapy could decrease thyroxine and worsen TSH increase in postmenopausal women with hypothyroidism treated with thyroxine[13]. Although in a randomized control trial involving 121 healthy women no relevant changes from baseline or differences between the groups were observed for TSH and thyroxine after 6 cycles use of combination oral contraceptives or progestin-only contraceptives. Both of them increased thyroxine-binding globulin, particularly for combination oral contraceptives [14]. These conflicting conclusions may result from the limitation of follow-up duration, sample size and various confounding factors. This research specially addressed these data gap.

The thyroid gland begins to develop as early as the first three months of pregnancy and is the first developing endocrine gland[15]. It is reported the maternal high estrogen environment is correlated with increased risk of fetal thyroid dysfunction, especially the elevated TSH levels[16]. The prevalence of hypothyroidism in women is 2-5 times that in men, implying hormones could be involved in the disease course[8 17]. Another study revealed TSH increased (3.0 vs. 2.3 mIU/L; P<0.0001) and free thyroxine (FT4) decreased (14.4 vs. 12.9 pmol/mlL; P<0.0001) significantly after controlled ovarian hyperstimulation, which were related to the rapid 10-fold estrogen increase[18]. Besides, an interplay

of early exposure to estrogens, as expressed by early menarche(P<0.0001), and full-term pregnancies (P=0.04) may be associated with hypothyroidism risk[19]. To minimize the confounding factors from other possible exposure of estrogen and progestin, we also calculated after adjusting for age, first menstrual period, pregnancy history, menopause status, medical use of hormones.

Normal function of thyroid mainly regulated by hypothalamus-pituitary-thyroid (HPT) axis[20]. Extra estrogen and progestogen introduced by long time use of OCs may disturb the hypothalamus-pituitary-gonadal axis as well as HPT axis, leading to dysfunction of thyroid[21]. Hyperestrogenemic states, including pregnancy, could promote the secretion of thyroxine-binding globulin to combine with FT4, thus decreasing the FT4 and elevating TSH through negative feedback[22]. Estrogen and progesterone could also influence iodine uptake whereas iodine-deficiency is the first cause of hypothyroidism[8]. Additionally, estrogen could down-regulate the expression of thyrotropin-releasing hormone mRNA in paraventricular nucleus cells and up-regulate the activity of thyroid peroxidase resulting in the decrement of thyroxine synthesis[23]. Moreover, estrogen may increase female susceptibility to thyroid disease by activation of the PI3K pathway in thyroid follicular cells[24]. Estrogen receptors are expressed in the majority of immune cells and estrogen can induce thyroid cell apoptosis, which may play a role in high incidence of thyroid auto-antibodies and autoimmune thyroid disease[25].

Though our results firstly reveal the significant association between history of taking birth control pills and hypothyroidism, but the specific medication of birth control use were unavailable in the NHANES, which is the main limitation of our study. Secondly, overt and subclinical hypothyroidism were not differentiated in our study for existed levothyroxine supplement. Finally, our results might not be appropriate for other regions.

In conclusion, our study used a large cohort of the US population to examine the association between a history of taking birth control pills and hypothyroidism. Longer history of taking birth control pills was strongly associated with hypothyroidism, especially more than 10 years. These findings have important implications for basic studies to determine whether there is a role for hypothyroid status and oral contraceptives.

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Figure Legends

Figure 1. Schematic representation of participant selection and distribution in participant groups

Figure 2. Forest plots of association between use of birth control pills and hypothyroid, and data presented as log odds ratio with 95% confidence interval: A, unadjusted; B, adjusted by model1; C, adjusted by model2; D, adjusted by model3. mo, month; yr, years

Table 1. Demographic and Clinical Characteristics of Study Population (N = 5,116)

Variable		No history N (weighted %)	History N (weighted %)	χ^2	P
Age	<=44	1050 (50.4)	1396 (46.0)	322.963	<0.001
	45-64	364 (17.5)	1153 (38.0)		
	<=65	668 (32.1)	485 (16.0)		
Race	Mexican American	412 (19.8)	465 (15.3)	87.845	<0.001
	Other Hispanic	280 (13.4)	334 (11.0)		
	Non-Hispanic White	792 (38.0)	1487 (49.0)		
	Non-Hispanic Black	417 (20.0)	610 (20.1)		
	Other Race	181 (8.7)	138 (4.5)		
Education	Less than high school diploma	540 (37.5)	664 (22.9)	135.868	<0.001
	High school diploma	347 (24.1)	622 (21.4)		
	Some college	337 (23.4)	955 (32.9)		
	College or more	215 (14.9)	661 (22.8)		
BMI status	Underweight	90 (4.4)	64 (2.1)	67.598	<0.001
	Normal	735 (35.9)	864 (28.8)		
	Overweight	581 (28.4)	865 (28.8)		
	Obese	640 (31.3)	1212 (40.3)		
Smoking status	Never	989 (68.5)	1707 (58.8)	44.868	<0.001
	Former	265 (18.4)	611 (21.1)		
	Current	189 (13.1)	584 (20.1)		
Alcohol use	Never or not in last 12 months	809 (61.9)	970 (36.7)	239.467	<0.001
	1 drink/day	230 (17.6)	617 (23.3)		
	2–3 drinks/day	186 (14.2)	793 (30.0)		
	4+ drinks/day	81 (6.2)	266 (10.1)		

First menstrual age	<10	55 (2.6)	115 (3.8)	14.332	0.002
	10 to 12	1017 (49.0)	1347 (44.4)		
	13 to 15	873 (42.0)	1386 (45.7)		
	>16	132 (6.4)	183 (6.0)		
Ever been pregnant	No	233 (16.2)	357 (12.4)	12.242	<0.001
	Yes	1203 (83.8)	2533 (87.6)		
Menopause status	No	1133 (54.5)	1650 (54.4)	0.004	0.951
	Yes	947 (45.5)	1384 (45.6)		
History of hormone use (not for birth control)	No	1111 (77.3)	2259 (78.1)	0.355	0.551
	Yes	326 (22.7)	633 (21.9)		
Self-reported history of thyroid disease	No	1119 (77.5)	2269 (78.4)	0.383	0.534
	Yes	324 (22.5)	626 (21.6)		
Current knowledge of thyroid disease	No	66 (20.6)	109 (17.8)	1.091	0.296
	Yes	254 (79.4)	503 (82.2)		
Thyroid status	Hyperthyroid	37 (1.8)	55 (1.8)	11.587	0.003
	Hypothyroid	294 (14.1)	536 (17.7)		
	Euthyroid	1751 (84.1)	2443 (80.5)		

Table 2. Association between use of birth control pills and hypothyroid

History	Event/Total (weighted %)	Unadjusted OR (95% CI)	P	Model1 OR (95% CI)	P	Model2 OR (95% CI)	P	Model3 OR (95% CI)	P
Any	294/2045 (14.4)	1.280 (1.104 - 1.484)	0.001	1.245 (1.043 - 1.486)	0.015	1.231 (0.749 - 2.025)	0.412	1.19 (0.717 - 1.976)	0.500
<1mo	21/206 (10.2)	0.715 (0.463 - 1.103)	0.129	0.758 (0.470 - 1.220)	0.254	0.576 (0.210 - 1.581)	0.284	0.545 (0.196 - 1.509)	0.243
1mo to 1yr	145/851 (17.0)	1.184 (0.961 - 1.459)	0.113	1.293 (1.021 - 1.636)	0.033	0.979 (0.520 - 1.842)	0.948	0.965 (0.507 - 1.834)	0.913
1 to 2 yrs	53/326 (16.3)	1.085 (0.795 - 1.480)	0.606	1.078 (0.768 - 1.512)	0.665	1.550 (0.532 - 4.509)	0.422	1.514 (0.511 - 4.487)	0.454
2 to 10 yrs	220/1207 (18.2)	1.329 (1.108 - 1.595)	0.002	1.262 (1.022 - 1.559)	0.030	1.224 (0.675 - 2.217)	0.506	1.158 (0.631 - 2.123)	0.636
>10 yrs	91/369 (24.7)	1.865 (1.440 - 2.415)	<0.001	1.555 (1.167 - 2.072)	0.003	4.025 (1.489 - 10.879)	0.006	3.837 (1.402 - 10.500)	0.009

Mo, month; yr, year

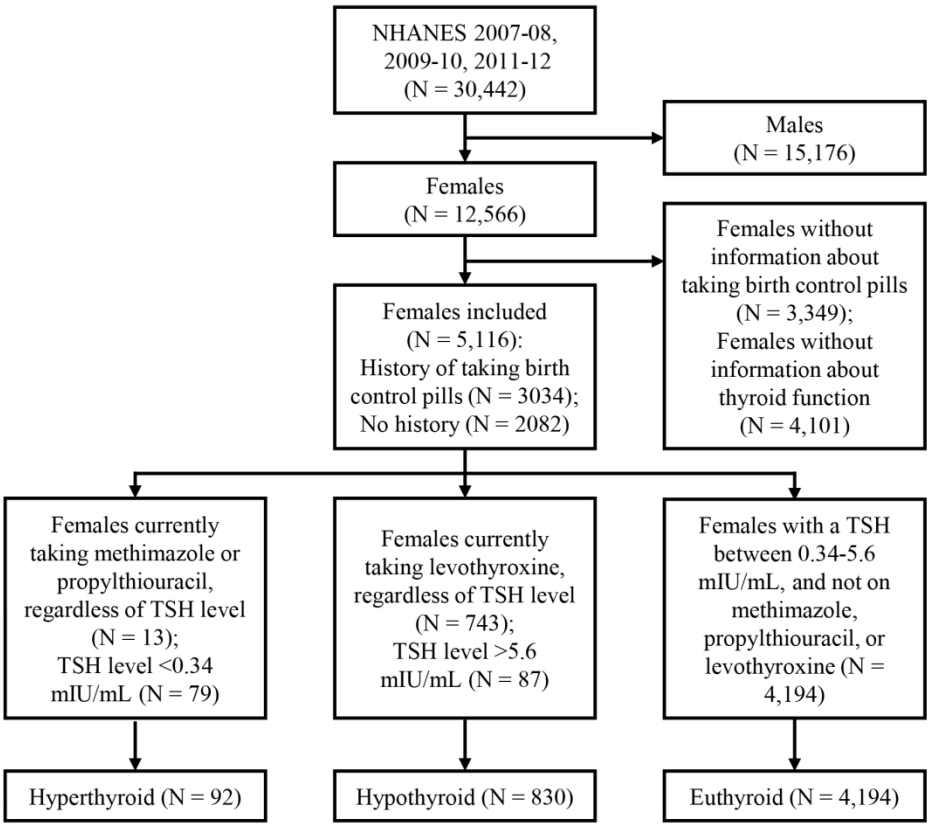

Figure 1. Schematic representation of participant selection and distribution in participant groups

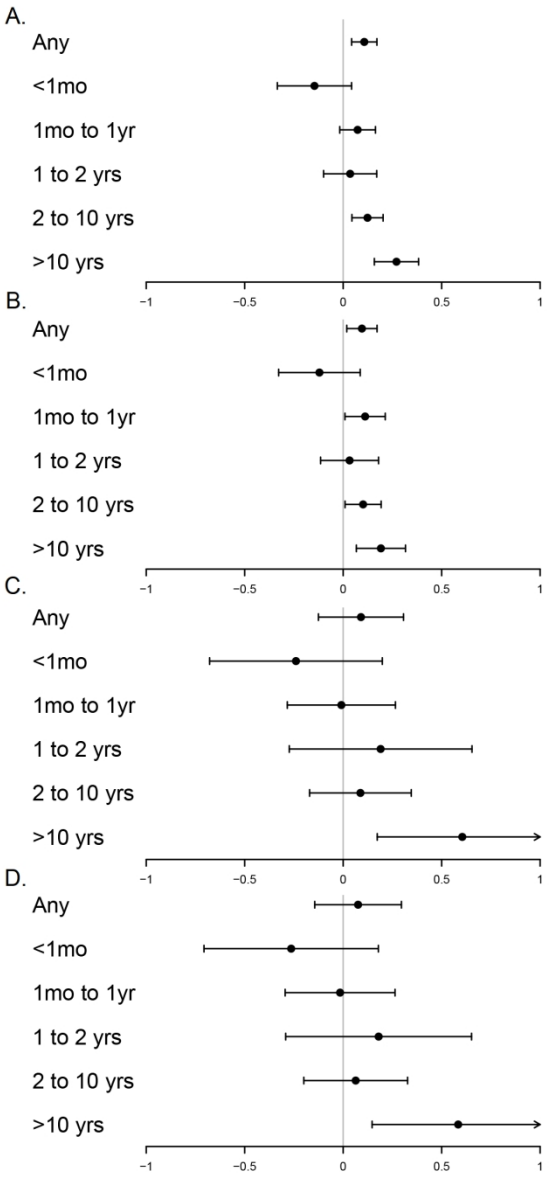

Figure 2. Forest plots of association between use of birth control pills and hypothyroid, and data presented as log odds ratio with 95% confidence interval: A, unadjusted; B, adjusted by model1; C, adjusted by model2; D, adjusted by model3. mo, month; yr, years

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Birth control pills and risk of hypothyroidism: a cross-sectional analysis of National Health and Nutrition Examination Survey (NHANES), 2007-2012

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Data sharing statement: All data were collected from National Health and Nutrition Examination Survey. We declare the authenticity and transparency of the data. Extra data can be available from e-mailing to Yuxuan Qiu.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethics approval: Ethical approval was part of National Health and Nutrition Examination Survey and no need in this study.

Informed consent: Participants got informed consents from National Health and Nutrition Examination Survey. All authors have read and approved the manuscript.

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Abstract

Objective The association between use of birth control pills and thyroid functions in women have not ever been well studied, but potential risk has been implicated by small sample-sized studies. We aimed to determine this association by large epidemiological surveys.

Design Cross-sectional study.

Setting National Health and Nutrition Examination Survey (NHANES) from 2007 to 2012.

Participants Female respondents aged 18+ who contained data about history of taking birth control pills and thyroid function were included. The history of taking birth control pills were based on responses in the reproductive health questionnaire. Participants not on antithyroid medication with TSH > 5.6 mIU/L and those on thyroid hormone replacement regardless of TSH were categorized as hypothyroid. Participants not on thyroid hormone replacement or antithyroid medication who had TSH between 0.34 and 5.6 mIU/L were classified as euthyroid.

Primary and secondary outcome measures The association between use of birth control pills and hypothyroidism based on multivariate logistic regression analysis.

Results A total of 5116 female adults with a history of taking birth control pills (n=3034) or not (n=2082) were included. Higher prevalence of hypothyroidism was found in those who have ever taken birth control pills (17.7% vs 14.1%; P=0.003). Multivariate logistic regression adjusted for cofounding covariables including age, race, education, body mass index, smoke status, alcohol use, self-reported history of thyroid disease, currently thyroid disease, first menstrual period, pregnancy history, menopause status, and history of hormone use demonstrated a significant association between history of taking birth control pills for more than 10 years and hypothyroidism (OR, 3.837; 95% CI, 1.402 to 10.500; P=0.009).

Conclusions Longer history of using birth control pills was strongly associated with hypothyroidism, especially for more than 10 years.

Key words: birth control pills, contraception, hypothyroidism, NHANES

Abbreviations: BMI, body mass index; CI, confidence interval; FT3, free triiodothyronine; FT4, free thyroxine; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; TSH, thyroid-stimulating hormone;

Article summary

Strengths and limitations of this study

- This study benefited from the large, nationally representative dataset and rigorous research methods of the National Health and Nutrition Examination Survey database.
- The study explored an association between oral contraception and hypothyroidism for the first time and controlled for important confounders.
- The limitations of this study were that our data were derived from cross-sectional studies, and the relationship is not necessarily identified as causal.
- Use of self-reported data might result in recall bias.

Introduction

Birth control pills have developed quickly and been widely used by an increasing number of women of child-bearing potential since its introduction[1]. As the most common form of effective and reversible contraception, the prevalence of birth control pills in women aged 15 to 45 was 17% among women and 27.3% among all contraception methods in the USA. Moreover, use of birth control pills declined as age increase: 54% of contraceptors were under 20 years old, 35% at 20 to 40 years old, and only 11% at age 40-45 years old[2]. Youth and popularization of birth control pills

warrants further investigation on safety, especially research into the long-term safety. Birth control pills were firstly designed for ovulation inhibition thus applying in birth control[3]. Over time, they not only helped avoiding unwanted pregnancies but also used in treatment for abnormal uterine bleeding, endometriosis, menstrual and hormonal disorder, etc. Additionally, long-term of taking birth control pills (≥10 years) could significantly decrease the risk of ovarian and endometrial cancer[4]. However, they could also bring many adverse effects including increase risk of hypertension, thromboembolic events, breast cancer, serious autoimmune diseases, and especially, endocrine related dysfunctions[5 6].

Thyroid hormone, one of the most notable endocrine hormones, is crucial for normal growth, energy metabolism and reproduction. Hypothyroidism is the most frequent pathological hormone insufficiency and may have a risk of high morbidity and mortality without treatment. It lacks specific symptoms at early stage but can lead to systemic symptoms such as chills and fatigue as the disease progresses and eventually present as myxedema or even heart failure. The prevalence of hypothyroidism was 4.6% in the USA according to the National Health and Nutrition Examination Survey (NHANES) III study. It accounts approximately 3-7 times higher in women compared to men, and its incidence raises with age[7 8]. Several drugs could cause hypothyroidism and the most notable were lithium, amiodarone and tyrosine kinase inhibitors[8]. However, considering its higher incidence in women, there may be an association between medication commonly used by women and thyroid function. A literature review summarized one cohort and two case-control studies and reported that use of birth control pills was linked to a lower incidence of hyperthyroidism and potentially higher risk of hypothyroidism[5 9].

In other words, the relationship between use of birth control pills and hypothyroidism was observed but existed studies were limited by their sample size and follow-up duration. We examined the NHANES database in representative of the US population to figure out whether a correlation exists between use of birth control pills and hypothyroidism.

Materials and methods

Patient and Public Involvement

We conducted a retrospective analysis of a cohort in the US population the National Health and Nutrition Examination Survey (NHANES), a periodic survey performed by National Center for Health Statistics (NCHS) with an informed consent to every participant. Therefore, there was no need for any ethical consent in this study. NHANES includes extensive demographic data, physical examinations, laboratory tests, health related questionnaires and lists of prescription medications. Data of NHANES 2007 to 2012 is the only continuous collection providing information of reproductive health questionnaires and laboratory tests of thyroid function in US women. We included women who offered information of taking birth control pills in the reproductive health questionnaire, reported thyroid medication use and had thyroid function laboratory test value. In the reproductive health questionnaire, the main questions were “Ever taken birth control pills?” and “How long taking birth control pills” and the choices were “yes; no; refused or don’t know” and the exact number of years, respectively. The knowledge of generic drug names was acquired in the prescription medications questionnaire and incidence of levothyroxine, methimazole and propylthiouracil were registered. Thyroid-stimulating hormone levels were available in thyroid profile testing by a 3rd generation, two-site immunoenzymatic (“sandwich”) assay (details see supplementary file).

Definitions of thyroid condition

Thyroid condition was estimated via reported currently taking medications and TSH testing in a manner similar to that of Thavaraputta et al.[10 11], which reported the prevalence of thyroid disease in the US by the diagnostic criteria. NHANES documentation provides a reference range of 0.34 to 5.6 mIU/L for normal TSH based on manufacturer's guidelines. Participants were defined as hyperthyroid if they reported currently taking methimazole or propylthiouracil, regardless of TSH level, or if their TSH level was <0.34 mIU/L. If the remaining participants reported currently taking levothyroxine regardless of TSH level, or if their TSH level was >5.6 mIU/L, they were defined as hypothyroid. Participants were defined as euthyroid if they were included in neither hyperthyroid nor hypothyroid.

Covariables and grouping

Demographic information on age, race/ethnicity and education was recorded at time of the interview. Body mass index (BMI) was coded into four categories based on standard cutoffs: underweight (<18.5 kg/m^2), normal BMI (18.5 to <25 kg/m^2), overweight (25 to <30 kg/m^2), and obese (≥30 kg/m^2). Smoking was coded as current, former, or never, and alcohol use was coded in four categories from never up to three or more drinks per day. Self-reported history and current knowledge of thyroid disease were included.

Participants were divided into two groups according to the reproductive health questionnaire whether they have ever taken birth control pills or not. If the participants had a history of taking birth control pills, they would be divided into history group; otherwise, they would be divided into no history group. Reproductive variables such as first menstrual period, pregnancy history, menopause status, history of hormone use were covered.

Statistical analysis

Statistical analyses were performed in StataSE 14.2 (StataCorp LLC, College Station). Chi-square tests were used in descriptive tables on population characteristics; multivariate logistic regression was used to estimate the odds of a hypothyroid diagnosis among participants with history of taking birth control pills. Coefficients of logistic regression models presented include an unadjusted model, followed by model 1, adjusting for demographic covariables including age, race, education, model 2, adding self-related covariables including BMI, smoke status, alcohol use, self-reported history of thyroid disease, currently thyroid disease, and model 3, continuously adding gynecological covariables including first menstrual period, pregnancy history, menopause status, history of hormone use and all variables from model 2. History of taking birth control pills were subgroups into history less than 1 month, 1 month to 1 year, 1 to 2 years, 2 to 10 years and >10 years. Statistical significance was set at $P < 0.05$.

Results

Population characteristics

The total number of participants in the 2007 to 2012 NHANES was 30442. Only 5116 female subjects met the inclusion criteria, including 2082 and 3034 women who never and ever taking birth control pills, respectively (Figure 1). Among the 3034 women who reported history of taking birth control pills, 210 (6.9%) have taken birth control pills less than 1 month, 864 (28.5%) have a history of 1 month and 1 year, 329 (10.8%) of 1 and 2 years, 1235 (40.7%) of 2 to 10 years, and 376 (12.4%) of longer than 10 years. Table 1 listed the demographics and health characteristics of the history group and no history group. Younger women (age < 65 years), non-Hispanic whites, participants with higher education, obese participants, currently smoking, higher alcohol consumption, history of pregnancy and later first menstrual period (age ≥ 13 years) had higher proportions of history

group than their counterparts. Menopause status, age of last menstruation and use of hormone including estrogen and progestin (not including birth control pills) were not different between two groups. Among the 5116 participants, 830 were identified as hypothyroid, 4194 as euthyroid, and 92 as hyperthyroid. Participants in history group were more frequently developing a hypothyroid status (17.7% vs 14.1%; P=0.003) with no difference in the history or current knowledge of thyroid disease.

Association between history of taking birth control pills and hypothyroid

According to univariate analysis, any history of taking birth control pills carried an odds ratio (OR) of 1.280 with a 95% confidence interval (CI) of 1.104 to 1.484 for developing hypothyroidism (P=0.001). Participants with a history of 2 to 10 years (OR, 1.329; 95% CI, 1.108 to 1.595; P=0.002) and >10 years (OR, 1.865; 95% CI, 1.440 to 2.415; P=0.000) were more likely to have a hypothyroidism diagnosis. After adjusting for model 1 (demographic covariables including age, race, education), any history of taking birth control pills remained high risk of hypothyroidism (OR, 1.245; 95% CI, 1.043 to 1.486; P=0.015). Participants with a history of 1 month to 1 year (OR, 1.293; 95%, 1.021 to 1.636; P=0.033), 2 to 10 years (OR, 1.262; 95% CI, 1.022 to 1.559; P=0.030) and >10 years (OR, 1.555; 95% CI, 1.167 to 2.072; P=0.003) were of higher risk of developing a hypothyroid status. However, after adjusting for model 2, adding self-related covariables including BMI, smoke status, alcohol use, self-reported history of thyroid disease, currently thyroid disease, women with a history of taking birth control pills for more than 10 years carried higher risk of hypothyroidism (OR, 4.025; 95% CI, 1.489 to 10.879; P=0.006). Similarly, after adjusting for model 3, adding gynecological covariables including first menstrual period, pregnancy history, menopause status, history of hormone use and all variables from model 2. women with a history of taking birth control pills for more than 10 years existed higher risk of hypothyroidism (OR, 3.837; 95% CI, 1.402 to 10.500; P=0.009). All details were displayed in Table 2.

Discussion

To the best of our knowledge, this is the first study revealed a strong association between long time use of birth control pills and hypothyroidism. Based on a large number of participants in NHANES, the incidence of hypothyroidism increased significantly along with the history of birth control pills use, even have adjusted. Participants with a history of 1 month to 1 year (OR, 1.293; 95% CI, 1.021 to 1.636; P=0.033), 2 to 10 years (OR, 1.262; 95% CI, 1.022 to 1.559; P=0.030) and >10 years (OR, 1.555; 95% CI, 1.167 to 2.072; P=0.003) were of higher risk of developing a hypothyroid status adjusting for demographic covariables including age, race, and education. A history of taking birth control pills for more than 10 years carried significantly higher risk of hypothyroidism (OR, 3.837; 95% CI, 1.402 to 10.500; P=0.009) after adjusting for all considered variates including age, race, education, BMI, smoke status, alcohol use, self-reported history of thyroid disease, currently thyroid disease, first menstrual period, pregnancy history, menopause status, and medical use of hormones. Birth control pills taking for over 10 years were burdened with higher susceptibility to hypothyroidism.

Some studies have investigated the relationship between birth control pills but conducted differently. In 1978, Frank et al. published the results of a cohort study of 23000 women currently taking contraceptive pills and a similar number of controls who never taken. It lasted for 14 months and indicated oral contraceptives exerted a protective effect against thyroid myxoedema with a relative risk of 0.57. Vestergaard et al. conducted a case-control study compromising 628 patients with autoimmune hypothyroidism and equal controls in a low-iodine intake area. It suggested that ever

use of oral contraceptives was associated with a slightly lower risk of Graves' disease in women, but not of autoimmune hypothyroidism[12]. Another case-control study conducted by Strieder et al. held opposite opinion that neither ever use (OR, 4.20; 95% CI, 0.55 to 32.43) nor current use (OR 0.89; 95% CI, 0.38 to 2.10) of oral contraception was associated with hypothyroidism[13]. A randomized control trial involving 121 healthy women were observed for TSH and thyroxine after 6 cycles use of combination oral contraceptives or progestin-only contraceptives and both groups increased thyroxine-binding globulin, particularly for combination oral contraceptives[14]. A retrospective study with 600 participants found oral contraceptive pills consumption was a significant risk factor in accelerating hypothyroidism in pregnant women (p=0.0004)[15]. These conflicting conclusions may result from the limitation of follow-up duration, sample size and various confounding factors. This research specially addressed these data gap.

Currently, there are quantity types of birth control pills available. Combination oral contraceptives containing both estrogen and progestin and progestin-only contraceptives are the two major types with many variations in the composition of the components[3]. Unfortunately, studies listed above failed to provide the details about birth control pills, so did this questionnaire-based cross-sectional analysis. The effects of progesterone or estrogen only on thyroid is less investigated and limited. Arafah et al. included 36 postmenopausal women with or without hypothyroidism and concluded a 12 weeks of estrogen therapy could decrease thyroxine and worsen TSH in postmenopausal women with hypothyroidism treated with thyroxine[16]. A 12-week randomized trial of oral micronized progesterone (progesterone, 300 mg/d at bedtime) conducted by Sathi et al. suggested free thyroxine (FT4) levels in were higher that placebo with TSH and free triiodothyronine (FT3) comparable[17]. Caufriez et al. found a reduction in TSH fluctuating with diurnal rhythmicity after a 3-week 300mg progesterone daily administration in 8 postmenopausal women. TSH concentrations kept a relatively stable daytime levels, followed by an early evening circadian rise, a nocturnal decrease, and a transient rebound after final morning awakening[18]. Those studies reveled a fluctuation in TSH but still far from the boundary value after a short intervention. It echoed with our study that short time birth control pills do not associated with hypothyroidism.

The thyroid gland begins to develop as early as the first three months of pregnancy and is the first developing endocrine gland[19]. It is reported the maternal high estrogen environment is correlated with increased risk of fetal thyroid dysfunction, especially the elevated TSH levels[20]. The prevalence of hypothyroidism in women is 2-5 times that in men, implying hormones could be involved in the disease course[8 21]. Another study revealed significantly increased TSH (3.0 vs. 2.3 mIU/L; P<0.0001) and decreased FT4 (14.4 vs. 12.9 pmol/mL; P<0.0001) after controlled ovarian hyperstimulation, which were related to the elevated estrogen and progesterone, especially the rapid 10-fold estrogen increase[22]. Besides, an interplay of early exposure to estrogens and progestins, as expressed by early menarche(P<0.0001), and full-term pregnancies (P=0.04) may be associated with hypothyroidism risk[23].

Normal function of thyroid mainly regulated by hypothalamus-pituitary-thyroid (HPT) axis[24]. Extra estrogen and progestogen introduced by long time use of OCs may disturb the hypothalamus-pituitary-gonadal axis as well as HPT axis, leading to dysfunction of thyroid[25]. Hyperestrogenemic states, including pregnancy, could promote the secretion of thyroxine-binding globulin to combine with FT4, thus decreasing the FT4 and elevating TSH through negative feedback[26]. Estrogen and progesterone could also influence iodine uptake whereas iodine-deficiency is the first cause of

hypothyroidism[8]. Additionally, estrogen could down-regulate the expression of thyrotropin-releasing hormone mRNA in paraventricular nucleus cells and up-regulate the activity of thyroid peroxidase resulting in the decrement of thyroxine synthesis[27]. While progesterone upregulated expression of thyroglobulin, thyroperoxidase, and sodium-iodide symporter mRNA in vitro[28]. Moreover, estrogen may increase female susceptibility to thyroid disease by activation of the PI3K pathway in thyroid follicular cells[29]. Estrogen receptors are expressed in the majority of immune cells and estrogen can induce thyroid cell apoptosis, which may play a role in high incidence of thyroid auto-antibodies and autoimmune thyroid disease[30].

In our study, a higher OR implied a higher risk of hypothyroidism as the extension of medication time. Hypothyroidism is a chronic pathophysiological process affected by inner and outer environmental balance. The internal environment homeostasis helps dealing with the changes including estrogen and progesterone administration through negative feedback. Therefore, a pathological thyroid won`t happen after a short time changes but occur under a long-time stimulation, such as taking birth control pills for over 10 years. The vast majority of cases of primary hypothyroidism were attributed to iodine deficiency and autoimmune disease (known as Hashimoto thyroiditis)[7 8 31]. Estrogen and progesterone are regarded as disruptors for iodine absorption and risk factors for Hashimoto thyroiditis. Most Hashimoto thyroiditis patients can maintain normal thyroid function for a long time, only a small number of them will show hyperthyroidism, and the rest will end with hypothyroidism[8 32 33].

To minimize the confounding factors from other possible exposure of estrogen and progestin, we calculated after adjusting for first menstrual period, pregnancy history, menopause status, medical use of hormones. Besides, the prevalence of TSH abnormalities increases with increasing age and with lower socioeconomic status; increasing age and lower socioeconomic status are also related to decreased progesterone production[34-36]. Occupation, overweight, smoking and alcohol are also significant risk factors to hypothyroidism[15 37]. Hence, we also considered the age, race, education, BMI, smoke status, and alcohol use[7].

Though our results firstly reveal the significant association between history of taking birth control pills and hypothyroidism, but the specific medication of birth control use were unavailable in the NHANES, which is the main limitation of our study. It`s accepted estrogen exerted more susceptibility to the hypothyroidism while progesterone on thyroid disorders merited further investigations. Secondly, overt and subclinical hypothyroidism were not differentiated in our study for existed levothyroxine supplement. Last but not least, the cross-sectional nature did not allow investigation of the causal relationship between birth control pills and hypothyroidism and this association might be affected by the recall nonresponse bias.

In conclusion, our study used a large cohort of the US population to examine the association between a history of taking birth control pills and hypothyroidism. Longer history of taking birth control pills was strongly associated with hypothyroidism, especially more than 10 years. These findings have important implications for basic studies to determine whether there is a role for hypothyroid status and oral contraceptives.

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Figure Legends

Figure 1. Schematic representation of participant selection and distribution in participant groups

Table 1. Demographic and Clinical Characteristics of Study Population (N = 5,116)

Variable		No history N (weighted %)	History N (weighted %)	χ^2	P
Age	<=44	1050 (50.4)	1396 (46.0)	322.963	<0.001
	45-64	364 (17.5)	1153 (38.0)		
	>=65	668 (32.1)	485 (16.0)		
Race	Mexican American	412 (19.8)	465 (15.3)	87.855	<0.001
	Other Hispanic	280 (13.4)	334 (11.0)		
	Non-Hispanic White	792 (38.0)	1487 (49.0)		
	Non-Hispanic Black	417 (20.0)	610 (20.1)		
	Other Race	181 (8.7)	138 (4.5)		
Education	Less than high school diploma	540 (37.5)	664 (22.9)	135.868	<0.001
	High school diploma	347 (24.1)	622 (21.4)		
	Some college	337 (23.4)	955 (32.9)		
	College or more	215 (14.9)	661 (22.8)		
BMI status	Underweight	90 (4.4)	64 (2.1)	67.598	<0.001
	Normal	735 (35.9)	864 (28.8)		
	Overweight	581 (28.4)	865 (28.8)		
	Obese	640 (31.3)	1212 (40.3)		
Smoking status	Never	989 (68.5)	1707 (58.8)	44.868	<0.001
	Former	265 (18.4)	611 (21.1)		
	Current	189 (13.1)	584 (20.1)		
Alcohol use	Never or not in last 12 months	809 (61.9)	970 (36.7)	239.467	<0.001
	1 drink/day	230 (17.6)	617 (23.3)		
	2–3 drinks/day	186 (14.2)	793 (30.0)		
	4+ drinks/day	81 (6.2)	266 (10.1)		

First menstrual age	<10	55 (2.6)	115 (3.8)	14.32[illegible]	0.002
	10 to 12	1017 (49.0)	1347 (44.4)		
	13 to 15	873 (42.0)	1386 (45.7)		
	>16	132 (6.4)	183 (6.0)		
Ever been pregnant	No	233 (16.2)	357 (12.4)	12.2[illegible]	<0.001
	Yes	1203 (83.8)	2533 (87.6)		
Menopause status	No	1133 (54.5)	1650 (54.4)	0.00[illegible]	0.951
	Yes	947 (45.5)	1384 (45.6)		
History of hormone use (not for birth control)	No	1111 (77.3)	2259 (78.1)	0.35[illegible]	0.551
	Yes	326 (22.7)	633 (21.9)		
Self-reported history of thyroid disease	No	1119 (77.5)	2269 (78.4)	0.38[illegible]	0.534
	Yes	324 (22.5)	626 (21.6)		
Current knowledge of thyroid disease	No	66 (20.6)	109 (17.8)	1.09[illegible]	0.296
	Yes	254 (79.4)	503 (82.2)		
Thyroid status	Hyperthyroid	37 (1.8)	55 (1.8)	11.58[illegible]	0.003
	Hypothyroid	294 (14.1)	536 (17.7)		
	Euthyroid	1751 (84.1)	2443 (80.5)		

Table 2. Association between use of birth control pills and hypothyroid

History	Event/Total (weighted %)	Unadjusted OR (95% CI)	P	Model 1 OR (95% CI)	P	Model 2 OR (95% CI)	P	Model 3 OR (95% CI)	P
Any	536/3034 (17.7)	1.280 (1.104 - 1.484)	0.001	1.245 (1.043 - 1.486)	0.015	1.231 (0.749 - 2.025)	0.412	1.19 (0.717 - 1.976)	0.500
<1mo	21/210 (10.0)	0.715 (0.463 - 1.103)	0.129	0.758 (0.470 - 1.220)	0.254	0.576 (0.210 - 1.581)	0.284	0.545 (0.196 - 1.509)	0.243
1mo to 1yr	145/864 (16.8)	1.184 (0.961 - 1.459)	0.113	1.293 (1.021 - 1.636)	0.033	0.979 (0.520 - 1.842)	0.948	0.965 (0.507 - 1.834)	0.913
1 to 2 yrs	53/329 (16.1)	1.085 (0.795 - 1.480)	0.606	1.078 (0.768 - 1.512)	0.665	1.550 (0.532 - 4.509)	0.422	1.514 (0.511 - 4.487)	0.454
2 to 10 yrs	220/1235 (17.8)	1.329 (1.108 - 1.595)	0.002	1.262 (1.022 - 1.559)	0.030	1.224 (0.675 - 2.217)	0.506	1.158 (0.631 - 2.123)	0.636
>10 yrs	91/376 (24.2)	1.865 (1.440 - 2.415)	<0.001	1.555 (1.167 - 2.072)	0.003	4.025 (1.489 - 10.879)	0.006	3.837 (1.402 - 10.500)	0.009

Mo, month; yr, year; Model 1, model adjusting for demographic covariables including age, race, and education; Model 2, model adjusting for self-related covariables including body mass index, smoke status, alcohol use, self-reported history of thyroid disease, and currently thyroid disease; Model 3, model adjusting for gynecological covariables including first menstrual period, pregnancy history, menopause status, history of hormone use and all variables from model 2.

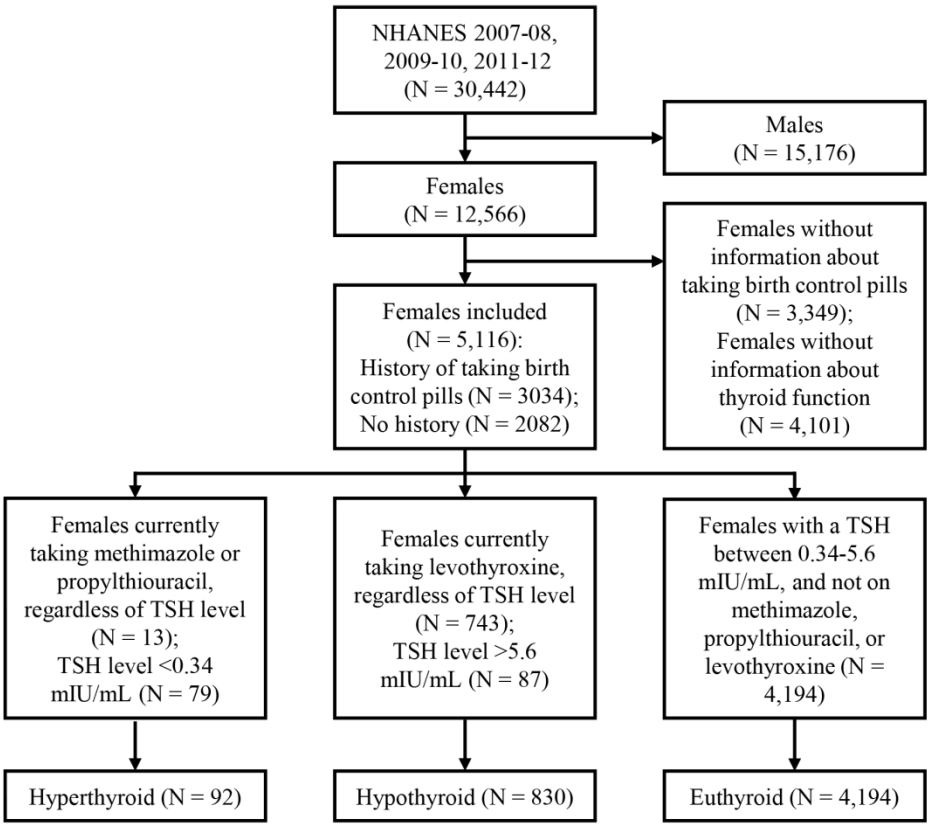

Figure 1. Schematic representation of participant selection and distribution in participant groups

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The Access HYPERsensitive hTSH Assay is a two-site immunoenzymatic ("sandwich") assay, for the quantitative determination of human thyroid-stimulating hormone in human serum, using the Access Immunoassay System. A sample is added to a reaction vessel with goat anti-hTSH-alkaline phosphatase conjugate, buffered protein solution, and paramagnetic particles coated with immobilized mouse monoclonal anti-hTSH antibody. (Goat anti-mouse antibody is used to immobilize the mouse anti-hTSH antibody.) The serum hTSH binds to the immobilized monoclonal anti-hTSH on the solid phase while the goat anti-hTSH-alkaline phosphatase conjugate reacts with a different antigenic site on the serum hTSH. Separation in a magnetic field and washing removes materials not bound to the solid phase. A chemiluminescent substrate, Lumi-Phos® 530, is added to the reaction vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of human thyroid-stimulating hormone in the sample. The amount of analyte in the sample is determined by means of a stored, multi-point calibration curve.

The major use of the hTSH assay is for the assessment of thyroid status. In patients with intact hypothalamic-pituitary function, hTSH is measured to: 1) exclude hypothyroidism or hyperthyroidism; 2) monitor T4 replacement treatment in primary hypothyroidism or antithyroid treatment in hyperthyroidism; 3) follow T4 suppression in "cold nodules" and non-toxic goiter; 4) assess the response to TRH stimulation testing. hTSH measurements are also used to identify subclinical and latent hypothyroidism or hyperthyroidism.

2. SAFETY PRECAUTIONS

Consider all plasma or serum specimens potentially positive for infectious agents including HIV and the hepatitis B virus. We recommend the hepatitis B vaccination series for all analysts working with whole blood and/or plasma. Observe universal precautions; wear protective gloves, laboratory coats. Place disposable plastic, glass, and paper (pipette tips, gloves, etc.) that contact plasma and any residual sample material in a biohazard bag and keep these bags in appropriate containers until disposal by maceration chlorination. Wipe down all work surfaces with Germicidal Disposable Wipe when work is finished.

Handle acids and bases with extreme care; they are caustic and toxic. Handle organic solvents only in a well-ventilated area or, as required, under a chemical fume hood.

Reagents and solvents used in this study include those listed in Section 6. Material safety data sheets (MSDSs) for these chemicals are readily accessible as hard copies in the lab.

3. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

A. Interferences:

1) No interference from 5-9 g/dL albumin, <10 mg/dL bilirubin or <1800 mg/dL triglycerides.

2) No interference from <500 mg/dL hemoglobin. Hemoglobin does

not affect the concentration of hTSH assayed.

B. Separated serum or plasma should not remain at +15°C to +30°C longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

C. Fasting is not required.

D. A minimum of 0.5 mL serum is needed for the TSH.

E. Sample volume for individual test is 110 µL.

F. Sample is run singly.

4. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

A. Instrumentation: Beckman Access2 Immunoassay System

B. Materials:

1) Access Immunoassay 1.0 mL Insert Cups (*Cat. #81915*)
2) Access Immunoassay 3.0 mL Sample Container (*Cat. #81914*)
3) Access Immunoassay Reaction Vessels (*Cat. #81901*)
4) Stockwell Scientific Tubes, 13x100mm, polystyrene, (Prod #8570)
5) S/P Plastic Transfer Pipette (*Cat. #P5214-10*)

C. Reagent Preparation:

1) Access HYPERsensitive hTSH Reagent Pack (*Cat. #33820*): 100 determinations, 50 tests/pack. Contains the following components:

R1a: Paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-hTSH complexes suspended in Tris buffered saline, with surfactant, bovine serum albumin (BSA), <0.1% sodium azide, and 0.1% ProClin™300.

R1b: Tris buffered saline with surfactant, BSA, protein (murine, goat), <0.1% sodium azide, and 0.1% ProClin™300.

R1c: Goat anti-hTSH-alkaline phosphatase (bovine) conjugate in Tris buffered saline, with surfactant, BSA, protein (goat), <0.1% sodium azide, and 0.1% ProClin™300.

a) Provided ready to use.
b) Store upright at 2-10°C.
c) Packs must be refrigerated at 2-10°C for two hours before loading on instrument.
d) Unopened packs are stable until expiration date when stored as directed.
e) After initial use, pack is stable for 28 days at 2-10°C.
f) CAUTION: Sodium azide may react with lead and copper plumbing. On disposal of liquid, flush drain with large volume of water. ProClin is a potential skin sensitizer, in

case of contact with reagent, thoroughly flush with water.

2) Access Substrate (*Cat. #81906*)

a) Lumi-Phos 530 (buffered solution containing dioxetane Lumigen PPD, flourescer, and surfactant).

b) Allow substrate to equilibrate, unopened at room temperature for a minimum of 18 hours (maximum 14 days) prior to use.

c) Unopened substrate is stable until expiration date when stored at 2-10°C

d) Opened substrate on board in external fluids tray is stable for 14 days.

e) Substrate is sensitive to air exposure. Keep tightly closed at all times. Do not pool bottles of substrate.

3) Access Wash Buffer (*Cat. #81907*).

a) Tris buffered saline, surfactant, 0.1% sodium azide and 0.1% ProClin 300.

b) Stable until expiration date when stored at room temperature.

D. Standards Preparation: No preparation required.

1) Beckman Access HYPERsensitive hTSH Calibrators (*Cat. #33825*).

E. Control Material:

1) Bio-Rad Immunoassay Plus Controls (Levels 1, 2, and 3) (*Cat. #371, 372, 373*).

a) Reconstitute each vial with 5 mL deionized water using a volumetric pipette. Replace the stopper and let control stand for 15 minutes. Before using, invert vial several times to mix.

b) Reconstituted control is stable for 7 days when stored at 2-8°C.

c) At least two levels of control should be analyzed in a 24-hour time period.

d) Ensure that assay control values are within the concentration ranges stated in the package insert or calculated from cumulative data at CLS.

e) Refer to Quality Control Flow Chart for action decision guidelines.

5. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibrators: Beckman Access HYPERsensitive hTSH Calibrators (*Cat. #33825*).

1) Six levels of calibrator.

2) Provided ready to use.

3) Mix contents by gently inverting prior to use.

4) Stable until expiration date when stored at 2-10°C.

5) Refer to calibration card enclosed with each set of calibrators for actual concentrations.

B. Calibration:

1) Calibration is required when a new lot of hTSH reagent is loaded,

when the calibration curve expires (curve stability is 28 days), or when controls are out of range.

2) Refer to Access2 Quick Reference Guide or Access2 "help" icon for detailed instructions on programming a calibration.

6. REPORTABLE RANGE OF RESULTS

A. Analytical Range:

1) 0.01 -The value of the highest calibrator (~100) µIU/mL.

2) A result over range high should be reported as ">100". To obtain a numerical answer, the specimen may be diluted one volume of sample to four volumes of 0.0 Calibrator or Access Sample Diluent A (*Cat. #81908*). After assaying the diluted sample, multiply the printed value by 5 to obtain the reportable answer.

3) Beckman defines sensitivity as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for the hTSH determination is 0.003 µIU/mL.

4) The literature suggests functional (clinical) sensitivity for hTSH assays is defined in terms of precision. Dose responses of 0.01-0.02 µIU/mL with interassay (between run) Cvs of ≤20% are considered to demonstrate "Third Generation" functional sensitivity performance.

5) CLS will periodically monitor low TSH reproducibility between runs by repeating patient samples. Previously repeated analysis within 1 day of samples with initial values between 0.01 and 0.03 yielded 8 results with no difference and two that differed by 0.01.

6) 0 is not a reportable value. Report results below 0.01 as <0.01.

7. QUALITY CONTROL (QC) PROCEDURES

A. Blind QC Specimens are included in the samples received from NHANES.

B. Bio-Rad Immunoassay Plus Controls levels 1, 2, 3 are assayed prior to running CDC-NHANES samples and after running CDC-NHANES samples.

C. Acceptable Answer:

1) Controls must be within ±2 S.D.

2) Refer to Quality Control Flow Chart for action decisions guidelines.

8. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

Remedial action for out of control conditions includes examination of the pipetting and detection equipment and examination of reagent materials. The QC parameters are compared to the patient means to look for confirmatory or disconfirmatory evidence. When the 2 2s and/or 1 3s rules are violated, samples are repeated following corrective maintenance or reagent changes.

9. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

A. Hemolyzed samples with up to 500 mg/dL hemoglobin have no significant interference.

B. <10 mg/dL bilirubin has no significant interference.

C. Lipemia has no significant interference in samples containing equivalent of 1800 mg/dL triglycerides.

D. Samples containing 5-9 g/dL (50-90 g/dL) albumin have no significant interference.

E. This assay has been formulated to minimize the effect of human anti-mouse antibodies or heterophile antibodies which may be present in some patient samples.

F. TSH levels obtained during the first trimester of pregnancy or whenever very high hCG levels are present should be interpreted with caution.

10. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens arrive frozen with dry ice. Specimens are kept frozen at -70°C until ready to analyze. Sample is thawed, mixed well by vortexing, and then transferred to sample cup or sample insert cup on the Access.

Specimen vials are returned to container and refrigerated after transfer of aliquot and double checking of Sample I.D. Specimen vial container is placed in -70°C freezer after testing is complete.

11. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

Samples will remain in -70°C freezer until instrument is back in operation.

More details see https://wwwn.cdc.gov/nchs/data/nhanes/2011-2012/labmethods/thyrod_g_met_tsh.pdf

STROBE Statement

Checklist of items that should be included in reports of observational studies

Section/Topic	Item No	Recommendation	Reported on Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	3
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4
		(*b*) Describe any methods used to examine subgroups and interactions	4
		(*c*) Explain how missing data were addressed	4
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	4
		(*e*) Describe any sensitivity analyses	4

Section/Topic	Item No	Recommendation	Reported on Page No
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4
		(b) Give reasons for non-participation at each stage	4
		(c) Consider use of a flow diagram	5
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5
		(b) Indicate number of participants with missing data for each variable of interest	5
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	5
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	5
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	5
		Cross-sectional study—Report numbers of outcome events or summary measures	5
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5
		(*b*) Report category boundaries when continuous variables were categorized	5
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	5
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	5
Discussion			
Key results	18	Summarise key results with reference to study objectives	5
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	7
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	6
Generalisability	21	Discuss the generalisability (external validity) of the study results	6
Other Information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

**Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.*

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Birth control pills and risk of hypothyroidism: a cross-sectional study of the National Health and Nutrition Examination Survey, 2007-2012

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Keywords:	Thyroid disease < DIABETES & ENDOCRINOLOGY, SEXUAL MEDICINE, General endocrinology < DIABETES & ENDOCRINOLOGY

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Birth control pills and risk of hypothyroidism: a cross-sectional study of the National Health and Nutrition Examination Survey, 2007-2012

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Abstract

Objective The association between use of birth control pills and thyroid functions in women have not ever been well studied, but potential risk has been implicated by small sample-sized studies. We aimed to determine this association by large epidemiological surveys.

Design Cross-sectional study.

Setting The National Health and Nutrition Examination Survey (NHANES) conducted in the US from 2007 to 2012.

Participants Female respondents aged 18+ who contained data about history of taking birth control pills and thyroid function were included. The history of taking birth control pills were based on responses in the reproductive health questionnaire. Participants not on antithyroid medication with TSH > 5.6 mIU/L and those on thyroid hormone replacement regardless of TSH were categorized as hypothyroid. Participants not on thyroid hormone replacement or antithyroid medication who had TSH between 0.34 and 5.6 mIU/L were classified as euthyroid.

Primary and secondary outcome measures The association between use of birth control pills and hypothyroidism based on multivariate logistic regression analysis.

Results A total of 5116 female adults with a history of taking birth control pills (n=3034) or not (n=2082) were included. Higher prevalence of hypothyroidism was found in those who have ever taken birth control pills (17.7% vs 14.1%; P=0.003). Multivariate logistic regression adjusted for cofounding covariables including age, race, education, body mass index, smoke status, alcohol use, history of thyroid disease, currently thyroid disease, first menstrual age, pregnancy history, menopause status, and history of hormone use demonstrated a significant association between history of taking birth control pills for more than 10 years and hypothyroidism (odds ratio, 3.837; 95% confidence interval, 1.402 to 10.500; P=0.009).

Conclusions Longer history of using birth control pills was strongly associated with hypothyroidism, especially for more than 10 years.

Key words: birth control pills, contraception, hypothyroidism, NHANES

Abbreviations: BMI, body mass index; CI, confidence interval; FT3, free triiodothyronine; FT4, free thyroxine; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; TSH, thyroid-stimulating hormone;

Article summary

Strengths and limitations of this study

- This study benefited from the large, nationally representative dataset and rigorous research methods of the National Health and Nutrition Examination Survey database.
- The study explored an association between oral contraception and hypothyroidism for the first time and controlled for important confounders.
- The limitations of this study were that our data were derived from cross-sectional studies, and the relationship is not necessarily identified as causal.
- Use of self-reported data might result in recall bias.

Introduction

Birth control pills have developed quickly and been widely used by an increasing number of women of child-bearing potential since its introduction[1]. As the most common form of effective and reversible contraception, the prevalence of birth control pills in women aged 15 to 45 was 17% among women and 27.3% among all contraception methods in the USA. Moreover, use of birth control pills declined as age increase: 54% of contraceptors were under 20 years old, 35% at 20 to

40 years old, and only 11% at age 40-45 years old[2]. Youth and popularization of birth control pills warrants further investigation on safety, especially research into the long-term safety. Birth control pills were firstly designed for ovulation inhibition thus applying in birth control[3]. Over time, they not only helped avoiding unwanted pregnancies but also used in treatment for abnormal uterine bleeding, endometriosis, menstrual and hormonal disorder, etc. Additionally, long-term of taking birth control pills (≥10 years) could significantly decrease the risk of ovarian and endometrial cancer[4]. However, they could also bring many adverse effects including increase risk of hypertension, thromboembolic events, breast cancer, serious autoimmune diseases, and especially, endocrine related dysfunctions[5 6].

Thyroid hormone, one of the most notable endocrine hormones, is crucial for normal growth, energy metabolism and reproduction. Hypothyroidism is the most frequent pathological hormone insufficiency and may have a risk of high morbidity and mortality without treatment[7]. It lacks specific symptoms at early stage but can lead to systemic symptoms such as chills and fatigue as the disease progresses and eventually present as myxedema or even heart failure. The prevalence of hypothyroidism was 4.6% in the USA according to the National Health and Nutrition Examination Survey (NHANES) III study. It accounts approximately 3-7 times higher in women compared to men, and its incidence raises with age[7 8]. Several drugs could cause hypothyroidism and the most notable were lithium, amiodarone and tyrosine kinase inhibitors[8]. However, considering its higher incidence in women, there may be an association between medication commonly used by women and thyroid function. A literature review summarized two studies and reported that use of birth control pills was linked to a potentially higher risk of hypothyroidism[9]. Strieder et al. reported ever use of contraceptives was possibly associated with hypothyroidism (relative risk [RR], 4.232; 95% confidence interval [CI], 0.552 to 32.425) in the case-control study enrolling 29 cases[10]. Besides, similar trend was confirmed by Frank et al. with a RR of 1.17 but a P-value of 0.552 in the cohort study with 47 cases[11].

In other words, the relationship between use of birth control pills and hypothyroidism was observed but existed studies were limited by their sample size and follow-up duration. We examined the NHANES database in representative of the US population to figure out whether use of birth control pills was associated with a higher risk of hypothyroidism.

Materials and methods

Patient and Public Involvement

We conducted a retrospective analysis of a cohort in the US population the National Health and Nutrition Examination Survey (NHANES), a periodic survey performed by National Center for Health Statistics (NCHS) with an informed consent to every participant. Therefore, there was no need for any ethical consent in this study. NHANES includes extensive demographic data, physical examinations, laboratory tests, health related questionnaires and lists of prescription medications. Data of NHANES 2007 to 2012 is the only continuous collection providing information of reproductive health questionnaires and laboratory tests of thyroid function in US women. We included women who offered information of taking birth control pills in the reproductive health questionnaire, reported thyroid medication use and had thyroid function laboratory test value. In the reproductive health questionnaire, the main questions were “Ever taken birth control pills?” and “How long taking birth control pills” and the choices were “yes; no; refused or don’t know” and the exact number of years, respectively. The knowledge of generic drug names was acquired in the prescription medications questionnaire and incidence of levothyroxine, methimazole and

propylthiouracil were registered. Thyroid-stimulating hormone levels were available in thyroid profile testing by a 3rd generation, two-site immunoenzymatic ("sandwich") assay (details in supplementary file).

Definitions of thyroid condition

Thyroid condition was estimated via reported currently taking medications and TSH testing in a manner similar to that of Thavaraputta et al.[12 13], which reported the prevalence of thyroid disease in the US by the diagnostic criteria. NHANES documentation provides a reference range of 0.34 to 5.6 mIU/L for normal TSH based on manufacturer's guidelines. Participants were defined as hyperthyroid if they reported currently taking methimazole or propylthiouracil, regardless of TSH level, or if their TSH level was <0.34 mIU/L. If the remaining participants reported currently taking levothyroxine regardless of TSH level, or if their TSH level was >5.6 mIU/L, they were defined as hypothyroid. Participants were defined as euthyroid if they were included in neither hyperthyroid nor hypothyroid.

Covariables and grouping

Demographic information on age, race/ethnicity and education was recorded at time of the interview. Body mass index (BMI) was coded into four categories based on standard cutoffs: underweight (<18.5 kg/m^2), normal BMI (18.5 to <25 kg/m^2), overweight (25 to <30 kg/m^2), and obese (≥30 kg/m^2). Smoking was coded as current, former, or never, and alcohol use was coded in four categories from never up to three or more drinks per day. The history and current knowledge of thyroid disease were included.

Participants were divided into two groups according to the reproductive health questionnaire whether they have ever taken birth control pills or not. If the participants had a history of taking birth control pills, they would be divided into history group; otherwise, they would be divided into no history group. Reproductive variables such as first menstrual age, pregnancy history, menopause status, history of hormone use were covered.

Missing Covariables

Addresses for 11% of the participants could not be geocoded and contributed to missing data in cross-sectional analyses. As such, 10 multiple imputations using fully conditional specification were used to address potential biases arising from item non-response.

Statistical analysis

Statistical analyses were performed in StataSE 14.2 (StataCorp LLC, College Station). Chi-square tests were used in descriptive tables on population characteristics; multivariate logistic regression was used to estimate the odds of a hypothyroid diagnosis among participants with history of taking birth control pills. Coefficients of logistic regression models presented include an unadjusted model, followed by model 1, adjusting for demographic covariables including age, race, education, model 2, all covariates from model 1 with individual covariables including BMI, smoke status, alcohol use, the history of thyroid disease, currently thyroid disease, and model 3, all covariates in model 2 with gynecological covariables including first menstrual age, pregnancy history, menopause status, history of hormone use and all variables from model 2. History of taking birth control pills were subgroups into history less than 1 month, 1 month to 1 year, 1 to 2 years, 2 to 10 years and >10 years. Statistical significance was set at P <0.05.

Patient and public involvement

The patient and public were not involved in the design of this study.

Results

Population characteristics

The total number of participants in the 2007 to 2012 NHANES was 30442. Only 5116 female subjects met the inclusion criteria, including 2082 and 3034 women who never and ever taking birth control pills, respectively (Figure 1). Among the 3034 women who reported history of taking birth control pills, 210 (6.9%) have taken birth control pills less than 1 month, 864 (28.5%) have a history of 1 month and 1 year, 329 (10.8%) of 1 and 2 years, 1235 (40.7%) of 2 to 10 years, and 376 (12.4%) of longer than 10 years. Table 1 listed the demographics and health characteristics of the history group and no history group. Younger women (age < 65 years), non-Hispanic whites, participants with higher education, obese participants, currently smoking, higher alcohol consumption, history of pregnancy or now pregnancy and later first menstrual age were of higher proportions of history of taking birth control pills than their counterparts. Menopause status, age of last menstruation and use of hormone including estrogen and progestin (not including birth control pills) were not different between two groups. Among the 5116 participants, 830 were identified as hypothyroid, 4194 as euthyroid, and 92 as hyperthyroid. Participants in history group were more frequently developing a hypothyroid status (17.7% vs 14.1%; P=0.003) with no difference in the history or current knowledge of thyroid disease.

Association between history of taking birth control pills and hypothyroid

According to univariate analysis, any history of taking birth control pills carried an odds ratio (OR) of 1.280 with a 95% confidence interval (CI) of 1.104 to 1.484 for developing hypothyroidism (P=0.001). Participants with a history of 2 to 10 years (OR, 1.329; 95% CI, 1.108 to 1.595; P=0.002) and >10 years (OR, 1.865; 95% CI, 1.440 to 2.415; P=0.000) were more likely to have a hypothyroidism diagnosis. After adjusting for model 1 (demographic covariables including age, race, education), any history of taking birth control pills remained high risk of hypothyroidism (OR, 1.245; 95% CI, 1.043 to 1.486; P=0.015). Participants with a history of 1 month to 1 year (OR, 1.293; 95%, 1.021 to 1.636; P=0.033), 2 to 10 years (OR, 1.262; 95% CI, 1.022 to 1.559; P=0.030) and >10 years (OR, 1.555; 95% CI, 1.167 to 2.072; P=0.003) were of higher risk of developing a hypothyroid status. However, after adjusting for model 2, adding individual covariables including BMI, smoke status, alcohol use, history of thyroid disease, currently thyroid disease, women with a history of taking birth control pills for more than 10 years carried higher risk of hypothyroidism (OR, 4.025; 95% CI, 1.489 to 10.879; P=0.006). Similarly, after adjusting for model 3, adding gynecological covariables including first menstrual age, pregnancy history, menopause status, history of hormone use and all variables from model 2, women with a history of taking birth control pills for more than 10 years existed higher risk of hypothyroidism (OR, 3.837; 95% CI, 1.402 to 10.500; P=0.009). All details were displayed in Table 2. The association between history of taking birth control pills and hypothyroid after excluding pregnant participants were shown in Table 3. Similarly, after adjusting for model 3, a history of taking birth control pills for more than 10 years was still associated with higher risk of hypothyroid (OR, 4.717; 95% CI, 1.721 to 12.926; P=0.003).

Discussion

To the best of our knowledge, this is the first study revealed a strong association between long time use of birth control pills and hypothyroidism. Based on a large number of participants in NHANES, the incidence of hypothyroidism increased significantly along with the history of birth control pills use, even have adjusted. Participants with a history of 1 month to 1 year (OR, 1.293; 95% CI, 1.021 to 1.636; P=0.033), 2 to 10 years (OR, 1.262; 95% CI, 1.022 to 1.559; P=0.030) and >10 years (OR, 1.555; 95% CI, 1.167 to 2.072; P=0.003) were of higher risk of developing a hypothyroid status

adjusting for demographic covariables including age, race, and education. A history of taking birth control pills for more than 10 years carried significantly higher risk of hypothyroidism (OR, 3.837; 95% CI, 1.402 to 10.500; P=0.009) after adjusting for all considered variates including age, race, education, BMI, smoke status, alcohol use, history of thyroid disease, currently thyroid disease, first menstrual age, pregnancy history, menopause status, and medical use of hormones. Birth control pills taking for over 10 years were burdened with higher susceptibility to hypothyroidism with or without excluding pregnant participants.

Some studies have investigated the relationship between birth control pills but conducted differently. In 1978, Frank et al. published the results of a cohort study of 23000 women currently taking contraceptive pills and a similar number of controls who never taken. It lasted for 14 months and indicated oral contraceptives exerted a protective effect against thyroid myxoedema with a relative risk of 0.57. Vestergaard et al. conducted a case-control study compromising 628 patients with autoimmune hypothyroidism and equal controls in a low-iodine intake area. It suggested that ever use of oral contraceptives was associated with a slightly lower risk of Graves' disease in women, but not of autoimmune hypothyroidism[14]. Another case-control study conducted by Strieder et al. held opposite opinion that neither ever use (OR, 4.20; 95% CI, 0.55 to 32.43) nor current use (OR 0.89; 95% CI, 0.38 to 2.10) of oral contraception was associated with hypothyroidism[10]. A randomized control trial involving 121 healthy women were observed for TSH and thyroxine after 6 cycles use of combination oral contraceptives or progestin-only contraceptives and both groups increased thyroxine-binding globulin, particularly for combination oral contraceptives[15]. A retrospective study with 600 participants found oral contraceptive pills consumption was a significant risk factor in accelerating hypothyroidism in pregnant women (P=0.0004)[16]. These conflicting conclusions may result from the limitation of follow-up duration, sample size and various confounding factors. This research specially addressed these data gap.

Currently, there are quantity types of birth control pills available. Combination oral contraceptives containing both estrogen and progestin and progestin-only contraceptives are the two major types with many variations in the composition of the components[3]. Unfortunately, studies listed above failed to provide the details about birth control pills, so did this questionnaire-based cross-sectional analysis. The prevalence of hypothyroidism in women is 2-5 times that in men, implying hormones could be involved in the disease course[8 17]. However, the effects of progesterone or estrogen only on thyroid is less investigated and limited. Arafah et al. included 36 postmenopausal women with or without hypothyroidism and concluded a 12 weeks of estrogen therapy could decrease thyroxine and worsen TSH in postmenopausal women with hypothyroidism treated with thyroxine[18]. A 12-week randomized trial of oral micronized progesterone (progesterone, 300 mg/d at bedtime) conducted by Sathi et al. suggested free thyroxine (FT4) levels in were higher that placebo with TSH and free triiodothyronine (FT3) comparable[19]. Caufriez et al. found a reduction in TSH fluctuating with diurnal rhythmicity after a 3-week 300mg progesterone daily administration in 8 postmenopausal women. TSH concentrations kept a relatively stable daytime levels, followed by an early evening circadian rise, a nocturnal decrease, and a transient rebound after final morning awakening[20]. Those studies reveled a fluctuation in TSH but still far from the boundary value after a short intervention. It echoed with our study that short time birth control pills do not associated with hypothyroidism. Another study revealed significantly increased TSH (3.0 vs. 2.3 mIU/L; P<0.0001) and decreased FT4 (14.4 vs. 12.9 pmol/mL; P<0.0001) with the elevated estrogen and

progesterone followed by controlled ovarian hyperstimulation, especially the rapid 10-fold estrogen increase[21].

Estrogen and progesterone could also influence iodine uptake whereas iodine-deficiency is the first cause of hypothyroidism[8]. Additionally, estrogen could down-regulate the expression of thyrotropin-releasing hormone mRNA in paraventricular nucleus cells and up-regulate the activity of thyroid peroxidase resulting in the decrement of thyroxine synthesis[22]. While progesterone upregulated expression of thyroglobulin, thyroperoxidase, and sodium-iodide symporter mRNA in vitro[23]. Moreover, estrogen may increase female susceptibility to thyroid disease by activation of the PI3K pathway in thyroid follicular cells[24]. Estrogen receptors are expressed in the majority of immune cells and estrogen can induce thyroid cell apoptosis, which may play a role in high incidence of thyroid auto-antibodies and autoimmune thyroid disease[25]. In order to minimize the confounding factors from other possible exposure of estrogen and progestin, we calculated after adjusting for first menstrual age, pregnancy history, menopause status, medical use of hormones.

In our study, a higher OR implied a higher risk of hypothyroidism as the extension of medication time. Hypothyroidism is a chronic pathophysiological process affected by inner and outer environmental balance. The internal environment homeostasis helps dealing with the changes including estrogen and progesterone administration through negative feedback. Therefore, a pathological thyroid won`t happen after a short time changes but occur under a long-time stimulation, such as taking birth control pills for over 10 years. The vast majority of cases of primary hypothyroidism were attributed to iodine deficiency and autoimmune disease (known as Hashimoto thyroiditis)[7 8 26]. Estrogen and progesterone are regarded as disruptors for iodine absorption and risk factors for Hashimoto thyroiditis. Most Hashimoto thyroiditis patients can maintain normal thyroid function for a long time, only a small number of them will show hyperthyroidism, and the rest will end with hypothyroidism[8 27 28].

The demographic characteristics were quite different between participants with or without history of taking birth control pills. Generally, the differences from baseline characteristics could contribute to the non-comparability of outcomes between the two groups. However, it is reported the prevalence of TSH abnormalities increased with older age and lower socioeconomic status[29-31]. That is to say, the difference of demographic characteristics could be associated with the development of hypothyroid status in our study. In addition, occupation, overweight, smoking and drinking are also significant risk factors to hypothyroidism[16 32]. Therefore, we took all the possible cofounders into account by multivariate logistic regression rather than matching the variants, for the fact that the latter could reduce the sample size.

Though our results firstly reveal the significant association between history of taking birth control pills and hypothyroidism, but the specific medication of birth control use were unavailable in the NHANES, which is the main limitation of our study. It`s accepted estrogen exerted more susceptibility to the hypothyroidism while progesterone on thyroid disorders merited further investigations. Secondly, overt and subclinical hypothyroidism were not differentiated in our study for existed levothyroxine supplement. Last but not least, the cross-sectional nature did not allow investigation of the causal relationship between birth control pills and hypothyroidism and this association might be affected by the recall nonresponse bias.

In conclusion, our study used a large cohort of the US population to examine the association between a history of taking birth control pills and hypothyroidism. Longer history of taking birth control pills was strongly associated with hypothyroidism, especially more than 10 years. These findings have

important implications for basic studies to determine whether there is a role for hypothyroid status and oral contraceptives.

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Competing interests: The authors declare that they have no conflict of interest.

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Data sharing statement: Data are available upon reasonable request.

Ethics approval: Ethical approval was not applicable in this study.

Informed consent: Patient consent was not applicable in this study.

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Figure Legends

Figure 1. Schematic representation of participant selection and distribution in participant groups

Table 1. Demographic and Clinical Characteristics of Study Population (N = 5,116)

Variable		No history N (weighted %)	History N (weighted %)	χ^2	P
Age	<=44	1050 (50.4)	1396 (46.0)	322.963	<0.001
	45-64	364 (17.5)	1153 (38.0)		
	>=65	668 (32.1)	485 (16.0)		
Race	Mexican American	412 (19.8)	465 (15.3)	87.855	<0.001
	Other Hispanic	280 (13.4)	334 (11.0)		
	Non-Hispanic White	792 (38.0)	1487 (49.0)		
	Non-Hispanic Black	417 (20.0)	610 (20.1)		
	Other Race	181 (8.7)	138 (4.5)		
Education	Less than high school diploma	540 (37.5)	664 (22.9)	135.868	<0.001
	High school diploma	347 (24.1)	622 (21.4)		
	Some college	337 (23.4)	955 (32.9)		
	College or more	215 (14.9)	661 (22.8)		
BMI status	Underweight	90 (4.4)	64 (2.1)	67.598	<0.001
	Normal	735 (35.9)	864 (28.8)		
	Overweight	581 (28.4)	865 (28.8)		
	Obese	640 (31.3)	1212 (40.3)		
Smoking status	Never	989 (68.5)	1707 (58.8)	44.868	<0.001
	Former	265 (18.4)	611 (21.1)		
	Current	189 (13.1)	584 (20.1)		
Alcohol use	Never or not in last 12 months	809 (61.9)	970 (36.7)	239.467	<0.001
	1 drink/day	230 (17.6)	617 (23.3)		
	2–3 drinks/day	186 (14.2)	793 (30.0)		
	4+ drinks/day	81 (6.2)	266 (10.1)		

First menstrual age	<10	55 (2.6)	115 (3.8)	14.332	0.002
	10 to 12	1017 (49.0)	1347 (44.4)		
	13 to 15	873 (42.0)	1386 (45.7)		
	>16	132 (6.4)	183 (6.0)		
Ever been pregnant	No	233 (16.2)	357 (12.4)	12.242	<0.001
	Yes	1203 (83.8)	2533 (87.6)		
Now pregnant	No	25 (9.3)	44 (4.8)	7.706	0.005
	Yes	243 (90.7)	871 (95.2)		
Menopause status	No	1133 (54.5)	1650 (54.4)	0.004	0.951
	Yes	947 (45.5)	1384 (45.6)		
History of hormone use (not for birth control)	No	1111 (77.3)	2259 (78.1)	0.354	0.551
	Yes	326 (22.7)	633 (21.9)		
History of thyroid disease	No	1119 (77.5)	2269 (78.4)	0.388	0.534
	Yes	324 (22.5)	626 (21.6)		
Current knowledge of thyroid disease	No	66 (20.6)	109 (17.8)	1.091	0.296
	Yes	254 (79.4)	503 (82.2)		
Thyroid status	Hyperthyroid	37 (1.8)	55 (1.8)	11.507	0.003
	Hypothyroid	294 (14.1)	536 (17.7)		
	Euthyroid	1751 (84.1)	2443 (80.5)		

Table 2. Association between use of birth control pills and hypothyroid

History	Event/Total (weighted %)	Unadjusted OR (95% CI)	P	Model 1 OR (95% CI)	P	Model 2 OR (95% CI)	P	Model 3 OR (95% CI)	P
Any	536/3034 (17.7)	1.280 (1.104 - 1.484)	0.001	1.245 (1.043 - 1.486)	0.015	1.231 (0.749 - 2.025)	0.412	1.190 (0.717 - 1.976)	0.500
<1mo	21/210 (10.0)	0.715 (0.463 - 1.103)	0.129	0.758 (0.470 - 1.220)	0.254	0.576 (0.210 - 1.581)	0.284	0.545 (0.196 - 1.509)	0.243
1mo to 1yr	145/864 (16.8)	1.184 (0.961 - 1.459)	0.113	1.293 (1.021 - 1.636)	0.033	0.979 (0.520 - 1.842)	0.948	0.965 (0.507 - 1.834)	0.913
1 to 2 yrs	53/329 (16.1)	1.085 (0.795 - 1.480)	0.606	1.078 (0.768 - 1.512)	0.665	1.550 (0.532 - 4.509)	0.422	1.514 (0.511 - 4.487)	0.454

2 to 10 yrs	220/1235 (17.8)	1.329 (1.108 - 1.595)	0.002	1.262 (1.022 - 1.559)	0.030	1.224 (0.675 - 2.217)	0.506	1.158 (0.631 - 2.123)	0.636
>10 yrs	91/376 (24.2)	1.865 (1.440 - 2.415)	<0.001	1.555 (1.167 - 2.072)	0.003	4.025 (1.489 - 10.879)	0.006	3.837 (1.402 - 10.500)	0.009

Mo, month; yr, year; Model 1, model adjusting for demographic covariables including age, race, and education; Model 2, model adjusting for individual covariables including body mass index, smoke status, alcohol use, history of thyroid disease, and currently thyroid disease and all variables from model 1; Model 3, model adjusting for gynecological covariables including first menstrual age, pregnancy history, menopause status, history of hormone use and all variables from model 2.

Table 3. Association between use of birth control pills and hypothyroid (Excluding pregnancy)

History	Event/Total (weighted %)	Unadjusted OR (95% CI)	P	Model 1 OR (95% CI)	P	Model 2 OR (95% CI)	P	Model 3 OR (95% CI)	P
Any	528/2916 (18.1)	1.310 (1.122 - 1.531)	0.001	1.562 (1.290 - 1.891)	<0.001	1.350 (0.815 - 2.238)	0.244	1.356 (0.805 – 2.284)	0.252
<1mo	21/203 (10.3)	0.680 (0.426 - 1.086)	0.107	0.898 (0.540 - 1.494)	0.680	0.492 (0.170 - 1.419)	0.189	0.506 (0.196 - 1.474)	0.212
1mo to 1yr	145/835 (17.4)	1.239 (0.996 - 1.540)	0.054	1.677 (1.303 – 2.160)	<0.001	1.025 (0.535 - 1.966)	0.940	1.011 (0.507 - 1.963)	0.974
1 to 2 yrs	52/320 (16.3)	1.144 (0.829 - 1.578)	0.414	1.411 (0.987 – 2.017)	0.059	1.640 (0.564 - 4.767)	0.364	1.694 (0.511 – 5.066)	0.346
2 to 10 yrs	219/1190 (18.4)	1.329 (1.097 - 1.611)	0.004	1.540 (1.227 - 1.933)	<0.001	1.318 (0.720 - 2.413)	0.371	1.306 (0.631 - 2.446)	0.404
>10 yrs	91/368 (24.7)	1.936 (1.482 - 2.530)	<0.001	1.926 (1.426 - 2.600)	<0.001	4.861 (1.785 - 13.241)	0.002	4.717 (1.721 - 12.926)	0.003

Mo, month; yr, year; Model 1, model adjusting for demographic covariables including age, race, and education; Model 2, model adjusting for individual covariables including body mass index, smoke status, alcohol use, history of thyroid disease, and currently thyroid disease and all variables from model 1; Model 3, model adjusting for gynecological covariables including first menstrual age, pregnancy history, menopause status, history of hormone use and all variables from model 2.

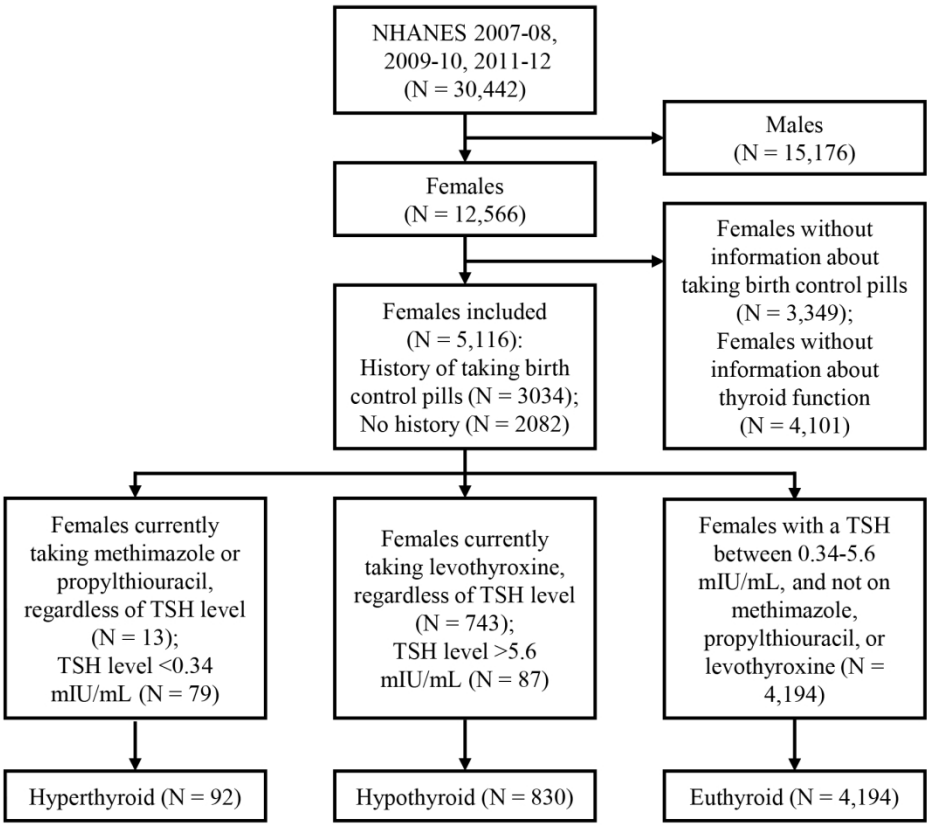

Figure 1. Schematic representation of participant selection and distribution in participant groups

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The Access HYPERsensitive hTSH Assay is a two-site immunoenzymatic ("sandwich") assay, for the quantitative determination of human thyroid-stimulating hormone in human serum, using the Access Immunoassay System. A sample is added to a reaction vessel with goat anti-hTSH-alkaline phosphatase conjugate, buffered protein solution, and paramagnetic particles coated with immobilized mouse monoclonal anti-hTSH antibody. (Goat anti-mouse antibody is used to immobilize the mouse anti-hTSH antibody.) The serum hTSH binds to the immobilized monoclonal anti-hTSH on the solid phase while the goat anti-hTSH-alkaline phosphatase conjugate reacts with a different antigenic site on the serum hTSH. Separation in a magnetic field and washing removes materials not bound to the solid phase. A chemiluminescent substrate, Lumi-Phos® 530, is added to the reaction vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of human thyroid-stimulating hormone in the sample. The amount of analyte in the sample is determined by means of a stored, multi-point calibration curve.

The major use of the hTSH assay is for the assessment of thyroid status. In patients with intact hypothalamic-pituitary function, hTSH is measured to: 1) exclude hypothyroidism or hyperthyroidism; 2) monitor T4 replacement treatment in primary hypothyroidism or antithyroid treatment in hyperthyroidism; 3) follow T4 suppression in "cold nodules" and non-toxic goiter; 4) assess the response to TRH stimulation testing. hTSH measurements are also used to identify subclinical and latent hypothyroidism or hyperthyroidism.

2. SAFETY PRECAUTIONS

Consider all plasma or serum specimens potentially positive for infectious agents including HIV and the hepatitis B virus. We recommend the hepatitis B vaccination series for all analysts working with whole blood and/or plasma. Observe universal precautions; wear protective gloves, laboratory coats. Place disposable plastic, glass, and paper (pipette tips, gloves, etc.) that contact plasma and any residual sample material in a biohazard bag and keep these bags in appropriate containers until disposal by maceration chlorination. Wipe down all work surfaces with Germicidal Disposable Wipe when work is finished.

Handle acids and bases with extreme care; they are caustic and toxic. Handle organic solvents only in a well-ventilated area or, as required, under a chemical fume hood.

Reagents and solvents used in this study include those listed in Section 6. Material safety data sheets (MSDSs) for these chemicals are readily accessible as hard copies in the lab.

3. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

A. Interferences:

1) No interference from 5-9 g/dL albumin, <10 mg/dL bilirubin or <1800 mg/dL triglycerides.

2) No interference from <500 mg/dL hemoglobin. Hemoglobin does

not affect the concentration of hTSH assayed.

B. Separated serum or plasma should not remain at +15°C to +30°C longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

C. Fasting is not required.

D. A minimum of 0.5 mL serum is needed for the TSH.

E. Sample volume for individual test is 110 µL.

F. Sample is run singly.

4. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

A. Instrumentation: Beckman Access2 Immunoassay System

B. Materials:

1) Access Immunoassay 1.0 mL Insert Cups (*Cat. #81915*)
2) Access Immunoassay 3.0 mL Sample Container (*Cat. #81914*)
3) Access Immunoassay Reaction Vessels (*Cat. #81901*)
4) Stockwell Scientific Tubes, 13x100mm, polystyrene, (Prod #8570)
5) S/P Plastic Transfer Pipette (*Cat. #P5214-10*)

C. Reagent Preparation:

1) Access HYPERsensitive hTSH Reagent Pack (*Cat. #33820*): 100 determinations, 50 tests/pack. Contains the following components:

R1a: Paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-hTSH complexes suspended in Tris buffered saline, with surfactant, bovine serum albumin (BSA), <0.1% sodium azide, and 0.1% ProClin™300.

R1b: Tris buffered saline with surfactant, BSA, protein (murine, goat), <0.1% sodium azide, and 0.1% ProClin™300.

R1c: Goat anti-hTSH-alkaline phosphatase (bovine) conjugate in Tris buffered saline, with surfactant, BSA, protein (goat), <0.1% sodium azide, and 0.1% ProClin™300.

a) Provided ready to use.
b) Store upright at 2-10°C.
c) Packs must be refrigerated at 2-10°C for two hours before loading on instrument.
d) Unopened packs are stable until expiration date when stored as directed.
e) After initial use, pack is stable for 28 days at 2-10°C.
f) CAUTION: Sodium azide may react with lead and copper plumbing. On disposal of liquid, flush drain with large volume of water. ProClin is a potential skin sensitizer, in

case of contact with reagent, thoroughly flush with water.

2) Access Substrate (*Cat. #81906*)

a) Lumi-Phos 530 (buffered solution containing dioxetane Lumigen PPD, flourescer, and surfactant).

b) Allow substrate to equilibrate, unopened at room temperature for a minimum of 18 hours (maximum 14 days) prior to use.

c) Unopened substrate is stable until expiration date when stored at 2-10°C

d) Opened substrate on board in external fluids tray is stable for 14 days.

e) Substrate is sensitive to air exposure. Keep tightly closed at all times. Do not pool bottles of substrate.

3) Access Wash Buffer (*Cat. #81907*).

a) Tris buffered saline, surfactant, 0.1% sodium azide and 0.1% ProClin 300.

b) Stable until expiration date when stored at room temperature.

D. Standards Preparation: No preparation required.

1) Beckman Access HYPERsensitive hTSH Calibrators (*Cat. #33825*).

E. Control Material:

1) Bio-Rad Immunoassay Plus Controls (Levels 1, 2, and 3) (*Cat. #371, 372, 373*).

a) Reconstitute each vial with 5 mL deionized water using a volumetric pipette. Replace the stopper and let control stand for 15 minutes. Before using, invert vial several times to mix.

b) Reconstituted control is stable for 7 days when stored at 2-8°C.

c) At least two levels of control should be analyzed in a 24-hour time period.

d) Ensure that assay control values are within the concentration ranges stated in the package insert or calculated from cumulative data at CLS.

e) Refer to Quality Control Flow Chart for action decision guidelines.

5. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibrators: Beckman Access HYPERsensitive hTSH Calibrators (*Cat. #33825*).

1) Six levels of calibrator.

2) Provided ready to use.

3) Mix contents by gently inverting prior to use.

4) Stable until expiration date when stored at 2-10°C.

5) Refer to calibration card enclosed with each set of calibrators for actual concentrations.

B. Calibration:

1) Calibration is required when a new lot of hTSH reagent is loaded,

when the calibration curve expires (curve stability is 28 days), or when controls are out of range.

2) Refer to Access2 Quick Reference Guide or Access2 "help" icon for detailed instructions on programming a calibration.

6. REPORTABLE RANGE OF RESULTS

A. Analytical Range:

1) 0.01 -The value of the highest calibrator (~100) µIU/mL.

2) A result over range high should be reported as ">100". To obtain a numerical answer, the specimen may be diluted one volume of sample to four volumes of 0.0 Calibrator or Access Sample Diluent A (*Cat. #81908*). After assaying the diluted sample, multiply the printed value by 5 to obtain the reportable answer.

3) Beckman defines sensitivity as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for the hTSH determination is 0.003 µIU/mL.

4) The literature suggests functional (clinical) sensitivity for hTSH assays is defined in terms of precision. Dose responses of 0.01-0.02 µIU/mL with interassay (between run) Cvs of ≤20% are considered to demonstrate "Third Generation" functional sensitivity performance.

5) CLS will periodically monitor low TSH reproducibility between runs by repeating patient samples. Previously repeated analysis within 1 day of samples with initial values between 0.01 and 0.03 yielded 8 results with no difference and two that differed by 0.01.

6) 0 is not a reportable value. Report results below 0.01 as <0.01.

7. QUALITY CONTROL (QC) PROCEDURES

A. Blind QC Specimens are included in the samples received from NHANES.

B. Bio-Rad Immunoassay Plus Controls levels 1, 2, 3 are assayed prior to running CDC-NHANES samples and after running CDC-NHANES samples.

C. Acceptable Answer:

1) Controls must be within ±2 S.D.

2) Refer to Quality Control Flow Chart for action decisions guidelines.

8. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

Remedial action for out of control conditions includes examination of the pipetting and detection equipment and examination of reagent materials. The QC parameters are compared to the patient means to look for confirmatory or disconfirmatory evidence. When the 2 2s and/or 1 3s rules are violated, samples are repeated following corrective maintenance or reagent changes.

9. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

A. Hemolyzed samples with up to 500 mg/dL hemoglobin have no significant interference.

B. <10 mg/dL bilirubin has no significant interference.

C. Lipemia has no significant interference in samples containing equivalent of 1800 mg/dL triglycerides.

D. Samples containing 5-9 g/dL (50-90 g/dL) albumin have no significant interference.

E. This assay has been formulated to minimize the effect of human anti-mouse antibodies or heterophile antibodies which may be present in some patient samples.

F. TSH levels obtained during the first trimester of pregnancy or whenever very high hCG levels are present should be interpreted with caution.

10. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens arrive frozen with dry ice. Specimens are kept frozen at -70°C until ready to analyze. Sample is thawed, mixed well by vortexing, and then transferred to sample cup or sample insert cup on the Access.

Specimen vials are returned to container and refrigerated after transfer of aliquot and double checking of Sample I.D. Specimen vial container is placed in -70°C freezer after testing is complete.

11. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

Samples will remain in -70°C freezer until instrument is back in operation.

More details see https://wwwn.cdc.gov/nchs/data/nhanes/2011-2012/labmethods/thyrod_g_met_tsh.pdf

STROBE Statement

Checklist of items that should be included in reports of observational studies

Section/Topic	Item No	Recommendation	Reported on Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	3
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4
		(*b*) Describe any methods used to examine subgroups and interactions	4
		(*c*) Explain how missing data were addressed	4
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	4
		(*e*) Describe any sensitivity analyses	4

Section/Topic	Item No	Recommendation	Reported on Page No
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4
		(b) Give reasons for non-participation at each stage	4
		(c) Consider use of a flow diagram	5
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5
		(b) Indicate number of participants with missing data for each variable of interest	5
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	5
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	5
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	5
		Cross-sectional study—Report numbers of outcome events or summary measures	5
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5
		(*b*) Report category boundaries when continuous variables were categorized	5
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	5
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	5
Discussion			
Key results	18	Summarise key results with reference to study objectives	5
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	7
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	6
Generalisability	21	Discuss the generalisability (external validity) of the study results	6
Other Information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

**Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.*

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.