BREASTFEEDING AND RISK OF METABOLIC SYNDROME IN CHILDREN AND ADOLESCENTS: A SYSTEMATIC REVIEW

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ABSTRACT

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Metabolic syndrome is an increasingly prevalent condition, in part due to rising obesity rates. Determining risk factors for metabolic syndrome is critical for primary prevention. Targeting early risk factors can slow the cascade of cardiometabolic risk factors that lead to metabolic syndrome. Among children and adolescents, not being breastfed is one potential risk factor for metabolic syndrome due to higher risk of obesity.

This systematic review assesses the association between being breastfed and the development of metabolic syndrome in children and adolescents. In 11 studies reviewed, seven found a protective association between breastfeeding and metabolic syndrome, and four failed to find an association. None of the studies found that being breastfed increased the risk of metabolic syndrome. There was no clear dose-response relationship between length of breastfeeding and metabolic syndrome risk and also no added effect of being exclusively breastfed. When rated on a quality assessment scoring system defined by the author, the overall quality of the articles was moderate. In general, lower quality articles failed to find an association, while higher quality articles did find an association. Odds ratios reported by higher quality articles tended to be closer to one or less, while lower quality articles had a wider range of odds ratios.

There is a lack of high quality research on the role of being breastfed and development of metabolic syndrome in children and adolescents. The evidence presented in this review implies that being breastfed may in fact be protective for metabolic syndrome in children and adolescents, but further research with improvements in study design is needed.

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KEY TO ABBREVIATIONS

AAP	American Academy of Pediatrics
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
CDC	Centers for Disease Control
CRF	Cardiorespiratory fitness
CVD	Cardiovascular disease
DL	Dyslipidemia
DM	Diabetes mellitus
EGIR	European Group for the Study of Insulin Resistance
FBG	Fasting blood glucose
GGT	Gamma glutamyl transferase
HBA1c	Glycated hemoglobin
HDL-C	High-density lipoprotein-cholesterol
HI	Hyperinsulinism
HOMA	Homeostasis Model Assessment
HT	Hypertension
IDF	International Diabetes Federation
IGT	Impaired glucose tolerance
LCPUFA	Long chain polyunsaturated fatty acid
LDL-C	Low-density lipoprotein-cholesterol

MetS	Metabolic syndrome
NCEP ATP	National Cholesterol Education Program Adult Treatment Panel
NEFA	Non-esterified fatty acids
OGTT	Oral glucose tolerance test
PRISMA	Preferred Reporting for Systematic Review and Meta Analyses
QUICKI	Quantitative Insulin Sensitivity Check Index
TC	Total Cholesterol
TG	Triglycerides
VLDL	Very low-density lipoprotein
WC	Waist circumference
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

Syndromes are conditions defined by a cluster of symptoms or abnormalities that are correlated with each other and are usually associated with a certain disease or disorder. Metabolic syndrome, which is associated with an increased risk for cardiovascular problems and diabetes, is defined by the presence of multiple risk factors, including central obesity, high triglyceride levels, low high-density lipoprotein cholesterol (HDL-C), hypertension, high fasting blood glucose levels, and insulin resistance [1,2]. In the United States (U.S.), the prevalence of metabolic syndrome is rising, indicating that increasing numbers of people are at risk for cardiovascular diseases [3]. Among children and adolescents, prevalence of metabolic syndrome has increased from 4.2% in 1988-1994 to 10.1% in 2001-2010 [4,5]. Current prevalence among adults is even higher than children and adolescents at 34.2% [6]. Since child and adolescent risk factors for metabolic syndrome are correlated with presence of metabolic syndrome in adulthood, reducing risk factors in children and adolescents can therefore lower adult prevalence [4,5,7].

Being breastfed has been shown to reduce the prevalence of many risk factors for metabolic syndrome in children and adolescents, including obesity, hypertension, high cholesterol, and diabetes [8-18]. Current recommendations from the American Academy of Pediatrics (AAP) and World Health Organization (WHO) include both duration and exclusivity of breastfeeding, both of which may be associated with metabolic syndrome. Recommendations are to exclusively breastfeed for six months, with continuation of breastfeeding for one year or as long as desired by the mother and infant [19,20]. Length of breastfeeding may be associated with metabolic syndrome due to evidence suggesting a dose-dependent relationship between length of

breastfeeding and decrease in risk of overweight among children [21], as well as lowered systolic blood pressure [22]. Exclusive breastfeeding, defined as an infant receiving only breast milk and no other liquids or solids except for drops or syrups consisting of vitamins, minerals, or medicines, is also associated with lower risk of high blood pressure and obesity in children and adolescents [8,22,23]. However, there is not consistent evidence connecting length or exclusivity of breastfeeding to other metabolic risk factors, such as high cholesterol and triglyceride levels [15]. Overall, there is evidence connecting breastfeeding to select risk factors of metabolic syndrome, but not conclusive evidence for metabolic syndrome as a whole.

The purpose of this review is to systematically evaluate evidence for the association between being breastfed and the later development of metabolic syndrome in children and adolescents. In addition to summarizing and reviewing results, quality of research must be considered. Low quality articles are subject to more forms of bias that may impact the validity of the results. For example, information bias (measurement bias) may occur. Information bias may occur if the components of metabolic syndrome are not measured correctly through incorrect instrumentation or misdiagnosis. This is especially problematic in the case of differential measurement bias where different rates of misclassification occur between those with and without metabolic syndrome. In this case, results may be biased towards finding an association in either direction (towards or away from the null hypothesis), whereas non-differential misclassification will bias the results towards the null. Recall bias and missing data are other examples of information bias. Recall bias may influence the participants reporting of the exposure. This may also bias the result in either direction. For example, a woman may report shorter breastfeeding due to health issues of the child. Missing data may cause bias if certain subjects are more likely to consistently have missing data. In addition, selection bias may result

in a non-representative sample of the population through poor sampling techniques or differential follow-up rates. This may bias the results in either direction. For instance, a subsample of the population may have greater baseline risk of metabolic syndrome, so the results may be more or less pronounced in either direction. Also, if certain subjects are more prone to dropping out of a study, this may also result in selection bias. In addition, confounding variables and effect modification may be altering the finding of a true association. Uncontrolled confounding variables may bias the results towards or away from the null, while controlling for an effect modifier may bias the results towards the null. To analyze sources of bias and validity of the results more objectively, a quality assessment scoring system was developed to assess the quality of the articles. The results of the articles will be reviewed in light of the quality assessment scores.

To begin, I discuss the mechanisms, risk factors, diagnosis, and implications of metabolic syndrome. Then, I review the proposed link between being breastfed and development of metabolic syndrome in children and adolescents. Finally, I review the significance and purpose of this review in more detail.

A. Metabolic syndrome

I. Mechanisms

There is no single etiology for metabolic syndrome; however, obesity and insulin resistance have been implicated as playing essential roles [24,25]. Obesity is strongly related to other metabolic risk factors, including hypertension, high serum cholesterol, low HDL-C, and hyperglycemia. In addition, obesity is a major cause of insulin resistance. Even mild-to-moderate weight gain can enhance the effects of insulin resistance [25].

When excess calories are consumed, triglycerides accumulate in hepatocytes, skeletal myocytes, and visceral adipocytes [26]. When there is excess adipose tissue, more nonesterified fatty acids (NEFA), cytokines, PAI-1, and adiponectin are released [25], which cause insulin resistance through down-regulation due to excess substrate availability [27,28]. The excess NEFA cause dysfunction of pancreatic beta cells, which are responsible for releasing insulin, resulting in the failure of the pancreas' ability to control glucose levels [28]. This accelerates the onset of glucose intolerance and the development of type 2 diabetes [24,28]. Excess NEFA also get diverted to the liver and cause an increase in secretion of glucose, triglycerides, and very low-density lipoproteins (VLDL), which can promote fatty liver and atherogenic dyslipidemia [25,27]. This cascade of events leads to increased likelihood of risk factors associated with development of metabolic syndrome.

II. Risk factors

As mentioned above, obesity and insulin resistance are central to metabolic syndrome and therefore are the primary risk factors [24,26,29]. In addition, genetic factors, such as an increased predisposition to central obesity and diabetes, increase the risk of metabolic syndrome [27,30]. Each metabolic syndrome component is also a risk factor (e.g., low HDL-C, high triglycerides), since an individual with more risk factors is more likely to be diagnosed with metabolic syndrome. Other risk factors include race or ethnicity, age, gender, and lifestyle factors. Hispanics have higher risk of metabolic syndrome in comparison to whites, who in turn have higher risk than blacks [31-33]. Blacks may have lower prevalence of metabolic syndrome due to favorable lipid profiles, despite higher prevalence of insulin resistance and obesity [34]. Metabolic syndrome risk also increases with age [31], and men are more likely to develop metabolic syndrome compared to women [32,33]. Lifestyle factors that affect metabolic

syndrome include exercise, smoking, alcohol use, and diet. Physical inactivity and smoking increase the risk of metabolic syndrome [31,33], while alcohol use is a more complicated risk factor. Mild to moderate drinking has been shown to decrease risk of metabolic syndrome, while heavy drinking increases risk [33,35,36]. Additionally, high carbohydrate and low fiber diets are a risk factor for metabolic syndrome [33,37].

III. Diagnosis

Since metabolic syndrome is a cluster of risk factors, it is defined as a syndrome. Diagnosis requires assessing the different risk factors associated with metabolic syndrome. Many different criteria have been proposed, as shown in Table A1 of Appendix A; each criterion requires the presence of usually at least three risk factors. Currently, the International Diabetes Federation (IDF) and National Cholesterol Education Program Adult Treatment Panel (NCEP ATP) III criteria, which focus on waist circumference as the central risk factor, are the most commonly used [38]. The IDF criteria were formulated in response to concerns that the existing criteria did not apply to different ethnic groups, while addressing both clinical and research needs. Because IDF criteria are formulated for worldwide use, these criteria are considered the standard definition of metabolic syndrome [38,39].

There is more opportunities for measurement error and misclassification when diagnosing metabolic syndrome because measurement of multiple risk factors is needed. This means that diagnosing each risk factor correctly is important. It is recommended that central obesity be measured as waist circumference instead of body mass index (BMI) because waist circumference better explains obesity-related health risk [40]. In addition, anthropometric measures should be measured multiple times to reduce measurement error. In research settings, waist circumference is generally measured three times to the nearest 0.1 cm [41]. It is also recommended that blood

pressure be measured multiple times per visit and after rest to avoid artificially high readings [42].

IV. Implications

Those with metabolic syndrome are at higher risk for many conditions, such as type 2 diabetes, atherosclerotic cardiovascular disease, coronary heart disease, and stroke [29]. It is estimated that metabolic syndrome doubles the risk for cardiovascular disease and increases the risk of type 2 diabetes by five-fold [43]. Additionally, metabolic syndrome is associated with an increased risk of mortality from coronary heart disease, cardiovascular disease, and all-cause mortality in adults [44,45]. Among children and adolescents, there is a dearth of literature on morbidity and premature mortality related to the metabolic syndrome. However, there is research on the components of the metabolic syndrome in children and adolescents, such as excess body weight. Child and adolescent overweight or obesity status is associated with an increase in premature mortality, as well as an increased risk of diabetes, hypertension, heart disease and stroke later in life [46,47]. Increased risk of morbidity and mortality may arise from the fact that those with metabolic syndrome have more metabolic and cardiovascular risk factors that predispose them to these conditions, and these risk factors make it harder for affected individuals to recover from cardiovascular events.

B. Being breastfed and metabolic syndrome in children and adolescents

Being breastfed has been linked to several components of metabolic syndrome, suggesting that breastfeeding is also related to metabolic syndrome as a whole. Children who are breastfed have been shown to have lower risk of obesity [8-13], improved blood pressure [14], improved cholesterol [15,16], and reduced risk of diabetes [17,18] compared to children not

breastfed. However, some studies have failed to establish consistent links between being breastfed and these factors [48-51].

Several possible biological mechanisms related to developmental programming may explain the association between being breastfed and metabolic syndrome. During critical periods in development, hormones, metabolites, and neurotransmitters program brain development and body functions, which affect disease risk later in life [52]. Developmental programming that occurs *in utero* and during infancy are both responsible for hormonal changes that persist through childhood. Breast milk can provide hormones, such as leptin, ghrelin, and adiponectin, which alter physiological processes that affect energy balance regulation [53]. These hormones influence energy balance regulation by altering glucose-insulin metabolism and hypothalamic development, thereby reducing excess weight gain [54-56]. Breast milk hormones are important in regulation of appetite, growth, and weight, and it has been suggested that breast milk hormones prevent obesity and affect food intake and food preferences later in life [57]. Additionally, these hormones are especially important in pre-term and low birth weight babies. In pre-term and low birth weight babies, consistent with the thrifty phenotype hypothesis, the fetus will make adaptations in order to maximize energy storage when subjected to maternal malnutrition [55]. This makes pre-term babies especially prone to rapid weight gain as a catch-up mechanism, which is associated with obesity in later life. Rapid weight gain can be attenuated by breast milk [58,59]. In comparison to breast milk, formula milk tends to have higher energy and protein content that can cause accelerated infant weight gain, which is associated with increased energy intake in childhood and reduced satiety, resulting in obesity [60-62].

It should be noted that being breastfed is associated with metabolic syndrome through other pathways besides obesity. Normal weight, yet metabolically obese individuals are

prevalent in the U.S. Among those with BMIs in a healthy range (18.5-24.9), prevalence was estimated to range from approximately one to nine percent in a graded fashion [63]. Therefore, there is utility in looking at the association of being breastfed and metabolic syndrome, and not solely obesity, since metabolic syndrome can occur in normal weight individuals. There are several possible mechanisms that may be responsible. First, the higher cholesterol content of breast milk has been implicated in regulating cholesterol synthesis, resulting in improved cholesterol levels in childhood and adolescence [64]. In addition, breast milk is also rich in long chain polyunsaturated fatty acids (LCPUFAs), which are protective against hypertension [65]. Breastfeeding may also affect metabolism through epigenetic changes. Nutrients transferred through breast milk alter metabolic phenotypes that are associated with risk of hypertension, impaired glucose homeostasis, and dyslipidemia [66].

C. Significance

Obesity prevalence has increased at an alarming rate, disproportionately affecting those of low socioeconomic status and minorities [67]. In 2011-2012, it was estimated that 16.9% of children and adolescents and 34.0% of adults were obese [68]. This rise in obesity brings concern to the subsequent rise in metabolic syndrome. Metabolic syndrome increases risk for cardiovascular disease, the leading cause of death in the U.S. that is responsible for 17% of health expenditures [69]. Reducing metabolic syndrome may reduce morbidity and mortality related to type 2 diabetes and cardiovascular disease.

Breastfeeding is an excellent target for an intervention because of its many benefits. In addition to the proposed link between breastfeeding and risk factors for metabolic syndrome, breastfeeding offers protection from both infectious and chronic diseases, such as respiratory tract infections, gastrointestinal tract infections, enterocolitis, allergies, celiac disease, and others

[70]. Providing a link between breastfeeding and metabolic syndrome may strengthen efforts to increase breastfeeding prevalence. In the U.S. from 2000 to 2008, overall breastfeeding prevalence increased from 70.3% to 74.6%. Additionally, the percentage of infants who are breastfed at six months increased, from 34.5% to 44.4% [71]. However, it was estimated in 2004 that only 11.3% of infants were exclusively breastfed through six months of age [72]. This is lower than estimates in Africa, Asia, and many other developing countries [73]. In addition, racial and ethnic disparities in breastfeeding practices persist in the U.S., with lower prevalence among blacks and higher prevalence among Hispanics and whites [71].

However, it should be noted that trends in breastfeeding rates in the U.S. have improved greatly in the past few decades. In 1989, rates were significantly lower than current rates. Only 52.2% of women initiated breastfeeding and only 18.1% exclusively breastfed at six months of age [74]. A decrease in metabolic syndrome prevalence should result from an increase in breastfeeding rates if breastfeeding is protective for metabolic syndrome. However, metabolic syndrome prevalence continues to climb despite increasing breastfeeding rates. This increase may be explained by the increase in other metabolic syndrome risk factors that breastfeeding alone cannot combat. For instance, dietary quality may be a factor. From 1989 to 2008, the intake of savory snacks, calzones, sweet snacks, candy, and fruit juice increased significantly in two to six year olds [75]. However, it has been noted that the intake of solid fats and sugars has leveled off in children and adolescents, but remains above the recommended levels [76]. Physical activity trends could exasperate the lack of dietary improvement. Although there is some evidence that physical activity levels have improved overall in children and adolescents [77], there are lifetime trends that are disconcerting. As adolescence progresses, physical activity decreases and computer time increases [78]. Another possible explanation is the rise in

prevalence of low birth weight babies. From 1990 to 2006, prevalence of low birth weight increased nearly 20% and has leveled off after slight decreases [79]. Low birth weight babies are at higher risk of metabolic syndrome particularly through higher triglyceride levels and higher blood pressure [80]. Lastly, the rise in prevalence of both type 1 and type 2 diabetes among children and adolescents may be contributing to the rise of metabolic syndrome [81]. Overall, breastfeeding may help lower risk for metabolic syndrome, but the rise of other potential risk factors may be offsetting progress towards lowering metabolic syndrome prevalence despite higher breastfeeding rates. In addition to improving breastfeeding rates, other potential interventions that target the rise of other risk factors may be implemented in order to stem the rise of metabolic syndrome prevalence.

If breastfeeding is in fact protective for metabolic syndrome, interventions that improve breastfeeding rates can target groups at risk for metabolic syndrome and also those with lower breastfeeding rates. In addition, guidelines for recommendations can be formed for use in clinical practice, with special importance placed on low birth weight and pre-term babies. This could potentially decrease the prevalence of metabolic syndrome in children and adolescents and future risk for cardiovascular disease (CVD) and diabetes, as well as provide other benefits such as nutrition and protection from diseases.

D. Purpose

The purpose of this thesis is to perform a systematic qualitative review of the relationship between being breastfed and the risk of metabolic syndrome in children and adolescents. To my knowledge, there are currently no review papers on this topic. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines are followed in the reporting of this review (Appendix B). My aims and hypotheses are as follows:

Aim 1: Summarize current evidence on the association between being breastfed and metabolic syndrome in children and adolescents to determine if sufficient evidence exists for continued research.

Hypothesis 1: Most (over half) of the studies in the review will have identified a statistically significantly inverse relationship between being breastfed and metabolic syndrome in children and adolescents.

Aim 2: Summarize the results by type and length of breastfeeding.

Hypothesis 2: Longer breastfeeding and exclusive breastfeeding will have a greater association with a reduction in risk of metabolic syndrome in children and adolescents compared with shorter breastfeeding and non-exclusive breastfeeding.

Aim 3: Determine if quality of articles affects the finding of an association between breastfeeding and metabolic syndrome in children and adolescents.

Hypothesis 3: Lower quality articles will be have less consistent results compared with higher quality articles due to sources of bias.

CHAPTER 2: METHODS

A. Search criteria

I searched PubMed and Embase for articles that investigated the association between being breastfed and the later development of metabolic syndrome in children and adolescents. The search terms "breastfeeding," "infant feeding," and "infant formula" were used in combination with the term "metabolic syndrome." Each page of results was searched for relevant articles. The abstracts of the search results were scanned for keywords and for relevance. The numbers of results obtained from each search ranged from 72 to 437 results per search, as shown in Table A2 of Appendix A. In addition to the PubMed and Embase searches, reference lists of the selected articles were searched to ensure that no relevant articles were missed.

Exploratory searches with the term "syndrome X" instead of "metabolic syndrome" did not improve results. The searches with the term "infant formula" did not return any useful results, but these searches were deemed important because articles may assess formula use instead of history of breastfeeding, although this did not occur.

B. Inclusion/exclusion criteria

Prior to the literature searches, inclusion criteria were determined. Due to the small number of articles published on this topic, inclusion criteria were designed to capture a wide array of studies. Multiple study designs, including cross-sectional, cohort, case-control, randomized controlled trials, or some combination of these designs, were accepted. Other study designs were not present among the relevant articles and thus were not included. The articles were required to be available in English, but no study population restriction was put in place. All relevant articles were published in 2007 and onward, so no time restriction was applied due to

recency of publication dates. Articles were excluded if they were review articles, letters, or conference abstracts. Only articles that assessed metabolic syndrome in children and adolescents ages three through 19 were included. This age range ensured that children and adolescents were both represented, and this is compliant with the definitions of children and adolescents used by the Centers for Disease Control (CDC) and Prevention and the World Health Organization (WHO) [82,83]. Articles that studied infants or adults out of this range were excluded. Metabolic syndrome as a whole was required to be an outcome of the study, not only one or some of the components of metabolic syndrome. For instance, if an article investigated the risk of high blood pressure and central obesity, but not metabolic syndrome as a whole, it was excluded. Since metabolic syndrome requires a combination of factors, as illustrated by the criteria in Table A1 of Appendix A, this increased the specificity of the search. Breastfeeding was not required to be the primary exposure of interest in the studies.

C. Quality assessment scores

The articles were assessed based on five categories: study design, missing information, detection of metabolic syndrome, breastfeeding data, and control of confounding variables. The purpose of this assessment was to determine how well the articles identified whether there was a relationship between being breastfed and development of metabolic syndrome in children and adolescents in order to address the specific aims of this review; this assessment does not evaluate overall general quality of the articles in relation to the authors' specific aims. Articles were judged on individual topics within each of the five categories and then received an average score for each category based on the following scale: 1=Poor, 2=Fair, 3=Moderate, 4=Good, or 5=Excellent. The average of the five categories was calculated for an overall score. In addition,

the results of the articles were reviewed in light of their quality assessment score to see if there were any emerging trends.

I. Category 1: Study design

The study design score was based on the following criteria:

- Study type: The articles were judged on which type of epidemiologic study design was used. Cohort studies were given a score of 5 because they are the gold standard of observational research. Cohort studies follow subjects through time, which may aid in proper assessment of exposure status and measurement of confounders that may vary by time. In addition, cohort studies are the preferred study design because measuring breastfeeding during infancy increases accuracy of reporting and reduces recall bias. Case-control studies and cross-sectional studies were given a score of 1 because the measurement of breastfeeding occurred years after breastfeeding cessation, so might be subject to recall bias. Cases and controls may recall exposure differently because knowledge of the disease may alter the individuals' perception and reporting of past exposures.
- Sample selection: Using a random sampling scheme aids in the selection of a non-biased sample of subjects. Articles were rated a score of 5 if a random sampling scheme was used to select the participants. If the authors failed to use a random sampling scheme, the articles were rated a score of 1. For case-control studies, however, articles were required to use a sampling scheme to select controls, not cases, from the population, as this is the general methodology used to conduct case-control studies.
- Population source: Number of sites, inclusion criteria, sample size, and location of recruitment (e.g. health centers) were evaluated. These components were assessed to

determine the generalizability of the results. The criteria for this category are as follows: recruitment from multiple locations, wide inclusion criteria, larger sample size, and recruitment from a more generalizable location. A more generalizable location in this case is a school or the community, instead of a specialty clinic or a randomized controlled trial. Subjects that are recruited from specialty clinics or from randomized controlled trials may be different from the general population, reducing generalizability of the results. Articles were rated a score of 5 if they met all these criteria. If studies did not meet just one of the criteria, they received a score of 4. If studies did not meet two of these criteria, they received a score of 3. If studies did not meet three of these criteria, they received a score of 2. If studies did not meet any of the criteria, they received a score of 1.

II. Category 2: Missing information

Missing information includes low participation rates, loss to follow-up and missing data. Participation rates, loss to follow-up, and missing data are measures that are not always reported. However, when they are reported, they are useful for interpretation of the results. For instance, subjects that do not enroll or complete a study may be inherently different than subjects that complete a study, which can cause biased results. Evaluating reasons for differential response and completion rates provides insight into the generalizability of the results. Loss to follow-up was only assessed in cohort studies because this is the only prospective study design. Otherwise, all studies were assessed regarding participation rates and missing data.

For each measure, the studies were scored based on whether they reported measures of participation rates, loss to follow-up, or missing data, the quality of the measure, and how missing information was handled. Measures were considered as high quality if rates were at 75%

or greater. High rates are an indication that the study methodology encouraged more complete information collection. If studies handled the missing information using statistical analyses to investigate potential implications, such as sensitivity analyses, comparison of subjects that completed the study and those that did not, or data imputation, they handled the missing information well. If the amount of missing information was less than 5%, the articles were not expected to investigate possible implications given low rates of missingness.

The scoring system was as follows: 5=included the measure, measure was of high quality, missing information handled well (if needed); 4=included the measure, measure was of poor quality, missing information handled well (if needed); 3=included the measure, measure was of good quality, missing information not handled well (if needed), 2=included the measure, measure was of poor quality, missing information not handled well (if needed); 1=did not include the measure.

III. Category 3: Detection of metabolic syndrome

The score for detection of metabolic syndrome was based on measurement of metabolic syndrome components as well as the quality of the measurements.

- Criteria included: As mentioned earlier, IDF guidelines are standard worldwide criteria for diagnosing metabolic syndrome. The articles will be graded on whether they collected the required components of this definition. This means that each article will be graded on whether they collected a measurement of central obesity, triglycerides, HDL-C, blood pressure, and glucose. In addition, the articles will be graded on whether they collected any disease or treatment history. For disease history, the articles were assessed on whether they collected information about diagnosis or treatment on at least one of the following: diabetes, lipid abnormalities, or hypertension. Articles received a score of 5 if

they collected all the necessary components. Articles received a 4 if they collected all but one component needed to diagnosis metabolic syndrome. Articles received a score of 3 if they collected all but two of the components. Articles received a score of 2 if they collected all but three of the components. Finally, articles received a score of 1 if they were missing four or more of the components.

- Quality of methods: Articles were rated based on how well components of metabolic syndrome were measured and also how well methods were reported. Articles received a 5 if investigators reported the following: the measurement of central obesity as waist circumference (instead of BMI), repeat measurements for central obesity, repeat measurements for blood pressure after a period of rest, fasting status of patients, assays used, and most of the instruments used (e.g. mercury sphygmomanometer, self-calibrating floor scale, stadiometer). Articles received a score of 4 if they missed one or two of these requirements. Articles received a score of 3 if they missed three of these requirements. Articles received a score of 2 if they missed four of these requirements. Lastly, articles received a score of 1 if they missed five or all of the requirements.

IV. Category 4: Breastfeeding data

For this category, the score was based on the following factors:

- Definition of breastfeeding: Clearly defining exposure is instrumental in determining casual relationship. Articles were rated a 5 if they measured duration of breastfeeding and recorded information on length of exclusive breastfeeding. Articles were rated a 4 if they either measured duration of breastfeeding or length of exclusive breastfeeding. Articles were rated a 3 if they measured length of breastfeeding, but they used less than three categories to define length. For example, if an article used “six months or less” and

"more than six months," the article was rated a 2. Articles were also rated a 2 if they assessed whether the child was ever breastfed, but within a specific time window. For instance, an article may describe breastfeeding as "ever breastfed in the first month." Articles were rated a 1 if they did not measure length or exclusive breastfeeding and simply recorded whether the child was ever breastfed.

- Breastfeeding data source: The validity of recalled breastfeeding duration has been shown to substantially decrease after three years of age [84]. Since the purpose of this study is to assess metabolic syndrome in children and adolescents, many of the articles collected data years after breastfeeding cessation. Collection of the data, either during examinations or self-report questionnaires throughout infancy, is the more reliable way to measure breastfeeding. Articles were rated a 5 if they collected breastfeeding information through these methods. Articles were rated a 3 if they retrospectively collected self-report breastfeeding data, but consulted medical histories. Articles were given a score of 1 if they retrospectively collected breastfeeding data, but did not consult medical records.

V. Category 5: Control of confounding variables

Control of confounding variables, either by including confounders in the final analysis or by matching subjects on confounders, was assessed. The studies were rated on the following categories of confounders:

- Child/adolescent demographics: Proper control of sex, race or ethnicity, and age was reviewed. Articles received a 5 if they controlled for all these factors. Articles received a 3 if they controlled for one or two of these factors. Articles received a 1 if they controlled for none of these factors.

- Maternal factors: Inclusion of maternal factors was assessed. Maternal BMI or weight, which has a U-shaped relationship with both breastfeeding and childhood metabolic syndrome risk [85-87], age, income, education, gestational diabetes, and smoking status during pregnancy were checked for inclusion. If articles controlled for at least four of these factors they received a score of 5. If they controlled for three they received a score of 4. If the articles controlled for two, they received a score of 3, and so on.
- Family history: Inclusion of family history of obesity, diabetes, hypertension, and an indicator of heart disease (e.g. cholesterol, history of heart attack) was assessed. If articles controlled for at least two of these, they received a score of 5. If they controlled one factor, they received a score of 3. If they controlled for none, they received a score of 1.
- Birth characteristics: Control for gestational age, birth order, and birth weight was assessed. If at least two of these factors were controlled for, the studies received a 5. If only one was controlled for, they received a score of 3. If none were controlled for, they received a score of 1.

CHAPTER 3: RESULTS

A. Description of selected studies

This review included a total of 11 studies. One study was a case-control study, three were cohort studies, and seven were cross-sectional studies. The publication dates ranged from 2007 to 2015. One study was conducted in Tunisia [88], one in Chile [89], two in China [90,91], three in Middle Eastern countries [92-94], and four in European countries [95-98]. None of the selected studies were conducted in the United States. Three of the studies sampled subjects from intervention trials addressing childhood obesity [92], iron supplementation [89], and the promotion of breastfeeding [98]. Two studies recruited subjects from an endocrinology department [93,96], one study recruited from a nephrology department [94], and one study recruited from a nutrition research unit [88]. The remaining four studies sampled subjects from schools [90,91,95,97]. The average sample size was 1,948 subjects, with a range of 84 to 13,616 subjects. The studies are described in more detail in Table A3 and Table A4 of Appendix A.

B. Results by breastfeeding definition

Overall, seven studies found a statistically significant inverse relationship between being breastfed and the development of metabolic syndrome in children and adolescents. Among these, reported odds ratios ranged from 0.08 to 0.39 [90,91,96,97]. None of the studies found that being breastfed increased the risk of metabolic syndrome. Four studies did not find a statistically significant association in either direction. The studies measured and defined breastfeeding in a variety of ways. Studies examined the risk of metabolic syndrome in relation to ever being breastfed, length of breastfeeding, length of exclusive breastfeeding, and participation in a breastfeeding promotion trial. The results of the studies are summarized in greater detail in Table

A5 of Appendix A.

I. Ever breastfed

Five studies investigated the association of ever being breastfed and development of metabolic syndrome [88,91,92,95,96]. One study specifically examined the relationship between ever being breastfed during the first six months of life [96]. Three of the five studies found an inverse relationship between ever being breastfed and development of metabolic syndrome [91,95,96]. Out of these five studies, two studies reported odds ratios, 0.32 (95% CI: 0.10, 0.97) and 0.08 (95% CI: 0.01, 0.72), respectively [91,96]. Two of the studies did not find a statistically significant relationship between ever being breastfed and metabolic syndrome, which did not report odds ratios [88,92].

II. Length of Breastfeeding

Length of breastfeeding was investigated in six studies [90,92,93,94,97,98]. Overall, three studies found an inverse relationship between length of breastfeeding and metabolic syndrome, and three found no association. One study compared the average length of breastfeeding between those with metabolic syndrome and those without metabolic syndrome and found it to be insignificant [93]. The other studies compared different breastfeeding groups (e.g. less than one month, one to three months). The categories were not consistent across studies, although some studies used similar categories. Six or more months of breastfeeding was associated with a reduced risk of metabolic syndrome in one study [92] and was not associated with risk of metabolic syndrome in two studies [94,98]. Out of these, only Martin et al. reported odd ratios. For their cluster and baseline-factor adjusted analysis, less than three months of breastfeeding was used as the reference group. Those who were breastfed three to less than six months had an odds ratio of 1.09 (95% CI: 0.86, 1.39), and those who were breastfed six months

of more had an odds ratio of 1.14 (95% CI: 0.68, 1.89) [98]. More than six months of breastfeeding was associated with a reduction of metabolic syndrome in two studies, with odds ratios of 0.13 (95% CI: 0.03, 0.65) and 0.39 (95% CI: 0.16, 0.98), respectively [90,97].

III. Exclusively breastfed for a period of time

The risk of metabolic syndrome among those who were exclusively breastfed was investigated in one study. This study found an inverse association between being exclusively breasted for three months and risk of metabolic syndrome, but did not report odds ratios [89].

IV. Participation in breastfeeding intervention trial

One study investigated the risk of metabolic syndrome in subjects who had participated in a trial that promoted breastfeeding. The study did not find a significant difference between those who were in the intervention arm and those in the control arm. In the cluster and baseline-factor adjusted analysis, an odds ratio of 1.16 (95% CI: 0.81, 1.66) was reported in the treatment arm that received the intervention [98].

C. Quality Assessment scores

Table 1: Quality assessment scores by category

First author (year)	Category 1: Study design	Category 2: Missing information	Category 3: Detection of metabolic syndrome	Category 4: Breastfeeding data	Category 5: Control of confounding	Total average score*
Ekelund (2009)	3.7	2.0	5.0	1.0	1.8	**2.7**
Esfarjani (2013)	2.7	1.0	4.0	2.0	3.8	**2.7**
Folic (2015)	2.7	1.0	4.0	1.5	2.8	**2.4**
González-Jiménez (2015)	2.3	1.0	3.5	3.0	2.5	**2.5**
Jamoussi (2012)	1.0	1.0	4.0	1.0	1.0	**1.6**
Khuc (2012)	4.3	3.3	3.0	5.0	2.5	**3.6**
Martin (2014)	4.3	3.7	3.0	5.0	2.3	**3.7**
Sen (2007)	1.3	1.0	4.0	2.5	1.0	**2.0**
Wang, J (2015)	3.3	4.5	4.5	2.5	2.8	**3.6**
Wang, S (2015)	3.7	1.0	3.5	1.0	3.3	**2.5**
Yakubov (2015)	2.3	1.3	2.5	4.5	1.0	**2.3**
Average	2.9	1.9	3.7	2.7	2.3	**2.7**

*1=Poor; 2=Fair, 3=Moderate, 4=Good, 5=Excellent

The quality assessment scores for each category are described below in Table 1. Overall, the articles received an average score of 2.7, with a range of 1.6 to 3.7. Tables A6-A10 of Appendix A describe the category scores in more detail. In the following sections, the reasoning for each score will be discussed.

I. Category 1: Study design

The average score for Category 1 was 2.9, with a range of 1.0 to 4.3. Three studies were cohort studies and received a score of 5 for the "Study type" topic area within this category [89,94,98]. The remaining studies received a score of 1, because they were either cross-sectional or case-control studies [88,90-93,95-97]. Seven articles received a score of 5 in the "Sample selection" topic area for using a random sampling scheme to select subjects [89,90,91,92,95,96,98], and four articles received a score of 1 for not using a random sampling scheme [88,93,94,97]. In the "Population source" topic area, three of the studies received a score of 5, due to selection of subjects from multiple schools or communities, larger sample sizes, inclusion of diverse subjects, and selection from a more generalizable location, which led to greater generalizability of the results [91,95,97]. Despite large sample size, multiple sampling locations, and recruiting from schools, one study received a score of 4 because the recruited subjects were a single ethnicity from a wealthy area, thereby decreasing generalizability [90]. Two studies received a 3 in this topic area because subjects were recruited from multiple sites and the studies had larger sample sizes; however, diversity of subjects was lacking, and the sample was biased because subjects were recruited from randomized controlled trials [89,98]. Three studies received a score of 2 in this area because they only met one of the criteria in this category [92,93,96]. The first of these studies recruited from nine health centers, which increased diversity of subjects, but had wide exclusion criteria, a small sample size, and recruited subjects

from an obesity prevention trial, which lowered diversity of subjects [92]. Another study had a small sample size, one location of recruitment, and recruited from an endocrinology department, but had wide inclusion criteria [96]. The last study recruited from one location in an endocrinology department and excluded many subjects, but had a moderate sample size [93]. The remaining two studies received a score of 1 in this category due to recruiting from one specialty clinic or division, low diversity of subjects, and small sample size [88,94].

II. Category 2: Missing information

The average score was 1.9 for this category, with a range from 1.0 to 4.5. For the first measure "Participation rate or response rate," one study received a score of 5 because a high participation rate of 97% was reported, thereby eliminating the need to address response attrition [98]. One article was assigned a score of 4 because the investigators reported a high response rate of 87%, but did not mention any methods used to handle the missing respondents [90]. One study received a score of 3 because a slightly lower response rate of 73% was reported, but researchers analyzed age and sex difference between respondents and non-respondents [92]. The remaining eight studies received a score of 1 because no participation rate was reported [88,89,91-94,96,97].

Only cohort studies were assessed for loss to follow-up. Two of the cohort studies received a score of 5 due to high rates of follow-up and analyses of the differences between those who had followed-up and those who did not [89,98]. One of these studies also performed a sensitivity analysis to determine if results were affected by loss to follow-up [98]. The other cohort study received a score of 1 because no follow-up rate was presented [94].

In terms of missing data, one study received a score of 5 because investigators reported a high percentage of subjects (97%) with complete data [90]. One study received a score of 4

because a high percentage of complete data was reported (93%), but no additional analyses were offered [89]. One study received a score of 2 because investigators reported that only 72% of subjects had complete data, but this was not addressed in their final analyses [94]. The remained studies received a score of 1 because the authors failed to report a measure for missing data [88,91-93,95-98].

III. Category 3: Detection of metabolic syndrome

The average score for this category was 3.7, with a range of 2.5 to 5.0. More details on detection of metabolic syndrome in each study are available in Table A11 and Table A12 of Appendix A.

First, I assessed which metabolic criteria were included. Three articles received a score of 5 for including all the aspects required to diagnose metabolic syndrome according to IDF criteria [93,95,96]. Seven articles received a score of 4 because all the anthropometric and biomarker tests required were included, but disease history was not recorded [88-92,94,97] One study received a score of 3, because researchers did not measure triglycerides and HDL-C [98].

Next, the quality and completeness of the methods were assessed. Two studies received a score of 5 because they reported measures of waist circumference instead of BMI, measured central obesity more than once, measured blood pressure more than once and after a period of rest, reported fasting status of patients, reported which assays were used, and reported most instruments that were used when diagnosing metabolic syndrome. Two studies received a score of 4 because they did not report one of these factors. For both studies, central obesity was not measured more than once [88,92]. Five studies received a score of 3 because three of the requirements were missed [91,93,96-98]. One study received a score of two due to missing four

requirements [89]. Finally, one study received a score of 1 because none of the requirements were filled [94].

IV. Category 4: Breastfeeding data

The average score for this category was 2.6, with a range of 1.0 to 5.0. More details on breastfeeding data collection are located in Table A13 of Appendix A.

First, the definition of breastfeeding was assessed. Two articles received a score of 5 because the studies assessed duration of breastfeeding and exclusivity of breastfeeding [89,98]. Four studies received a score of 4 because information was collected on duration of breastfeeding by months, but exclusive breastfeeding was not mentioned [90,93,94,97]. One study received a score of 3 because length of breastfeeding was assessed but breastfeeding was only described as breastfeeding less than six months or six months or more, and exclusive breastfeeding was not mentioned [92]. One study received a score of 2 because they assessed breastfeeding ever during the first six months of life and failed to mention exclusive breastfeeding [96]. The remaining three studies received a score of 1 because they only measured breastfeeding as ever breastfed [88,91,95].

Next, the breastfeeding data source was rated. Three articles received a score of 5 because they had collected breastfeeding data in real-time, either via questionnaire or during a physical exam [89,94,98]. One article received a score of 3 because breastfeeding data was collected by self-report retrospectively, but investigators checked past medical history [97]. The remaining seven articles received a score of 7 because they had collected breastfeeding only by self-report retrospectively [88,90-93,95].

V. Category 5: Control of confounding variables

The average score for this category is 2.3, with a range of 1.0 to 3.8. More information on covariates collected in each study is located in Table A14 of Appendix A.

One study received a score of 5 for control of demographics because sex and age were included in multivariate analysis. All the subjects were ethnic Han so ethnicity did not need to be included in analyses [90]. Six studies received a 3 because researchers controlled for two of the demographic characteristics [89,91,92,95,97,98]. The remaining four studies received a score of 1 because sex, race or ethnicity, and age were not controlled for [88,93,94,96].

As for maternal factors, none of the studies controlled for at least four of the maternal factors, so no article received a score of 5. Two studies received a score of 4 because they controlled for three of the maternal factor variables listed [92,96]. One study received a score of 3 for controlling for two of these factors [97]. Four studies received a score of 2 for controlling for one of the maternal factors [90-92,98]. Four studies received a score of 1 for not controlling for any of the maternal factors [88,89,93,94].

Family history of obesity, diabetes, hypertension, and heart disease were evaluated next. Two studies received a score of 5 because they included family history of two of these diseases was included [89,96]. Two studies received a score of 3, because they included family history for one of the diseases [91,92]. The remaining seven studies received a score of 1 because they did not include any family history for these diseases [88,90,93-95,97,98].

Control for birth factors, including gestational age, birth weight, and birth order was assessed next. Two studies received a score of 5 because they controlled for two out of three of these factors [91,92]. Three studies received a score of 3 because the authors controlled for birth

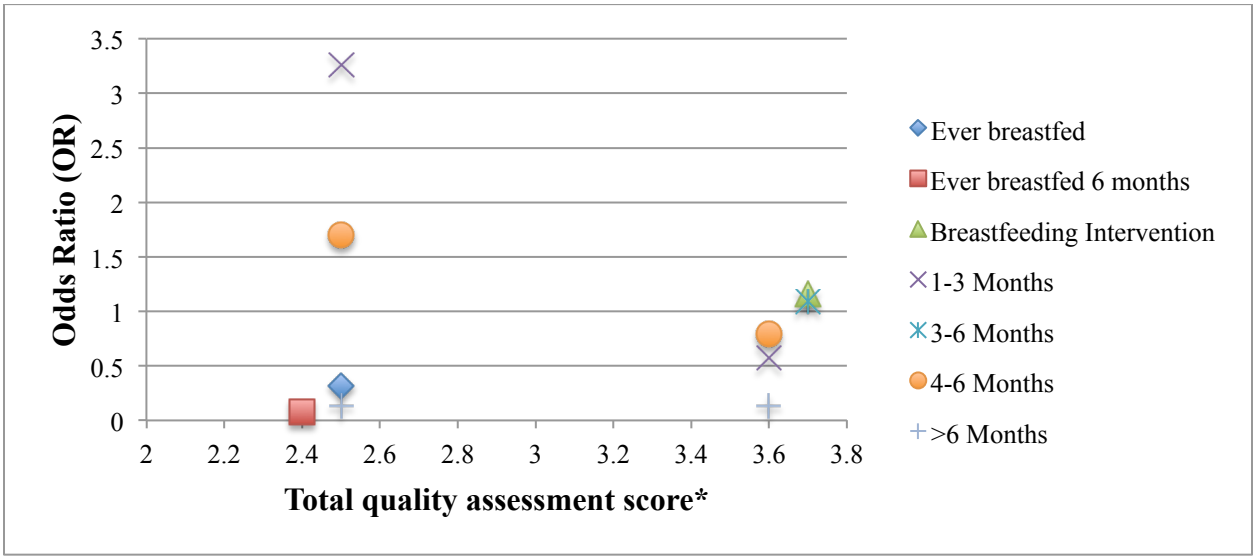
weight, but not the other two factors [89,90,97]. Finally, five studies received a score of 1 because they did not control for any of these birth factors [88,89,93-96].

D. Evidence by quality of study

Figure 1 summarizes the odds ratios that were reported. For Martin et al., only cluster-adjusted and baseline adjusted odds ratios were reported for simplicity.

As breastfeeding was hypothesized to be protective for metabolic syndrome, odds ratios of less than one were expected. Three out of five odds ratios reported by higher quality articles met this expectation, while the other two odds ratios were above one. The lower quality studies had a larger range of odds ratios, suggesting that there was less agreement among lower quality articles. There appeared to be no patterns regarding length of breastfeeding in relation to strength of odds ratios.

Figure 1: Odds ratios reported, by breastfeeding subgroup and quality assessment score



*1=Poor; 2=Fair, 3=Moderate, 4=Good, 5=Excellent

Table 2 summarizes the studies by rating and whether they had found a significant protective association of breastfeeding and metabolic syndrome. Most articles that failed to find a statistically significant inverse association were clustered towards the bottom of the rating scale

[88,93,94] indicating that worse quality articles were less likely to find an association. However, the article with the highest rating did not find a statistically significant protective association [98].

Table 2: Article results by quality assessment score

Article	Quality assessment score	Rating	Found protective association
Martin (2014)	3.7	Good	N
Khuc (2012)	3.6	Good	Y
Wang, J (2015)	3.6	Good	Y
Ekelund (2009)	2.7	Moderate	Y
Esfarjani (2013)	2.7	Moderate	Y
González-Jiménez (2015)	2.5	Fair-Moderate	Y
Wang, S (2015)	2.5	Fair-Moderate	Y
Folic (2015)	2.4	Fair	Y
Yakubov (2015)	2.3	Fair	N
Sen (2007)	2.0	Fair	N
Jamoussi (2012)	1.6	Fair	N

CHAPTER 4: DISCUSSION

A. Summary of findings

The articles included in this review present results from cross-sectional, cohort, and case-control studies examining the associations between being breastfed and later development of metabolic syndrome in childhood or adolescence. Most studies were cross-sectional, and all were conducted in countries outside the United States (U.S.). The majority of the studies (7 out of 11), found a protective association between being breastfed and metabolic syndrome in children and adolescents, while none of the studies included in this review found that being breastfed increased the risk of metabolic syndrome. Overall, these findings provide further evidence for an inverse association between breastfeeding and metabolic syndrome and suggest additional directions for research.

Three out of five articles that investigated ever being breastfed and development of metabolic syndrome found an inverse association. Three out of six that investigated length of breastfeeding and metabolic syndrome found an inverse relationship. Although individually some studies found a dose-response relationship when investigating length of breastfeeding [90,97], there was no clear trend when looking at all articles jointly. Only one study investigated the association between exclusive breastfeeding and metabolic syndrome, which found a significant inverse association between exclusive breastfeeding for three months and development of metabolic syndrome [89]. However, since only one study included information on exclusive breastfeeding, additional research is needed to determine if a relationship between exclusive breastfeeding and metabolic syndrome truly exists. Lastly, one article assessed the effect of a breastfeeding intervention, the Promotion of Breastfeeding Intervention Trial (PROBIT) on metabolic syndrome [89]. The intervention was modeled on the Baby Friendly

Hospital Initiative and consisted of health care worker assistance initiating and maintaining breastfeeding and lactation, and providing breastfeeding support [99]. The authors suggested that they failed to find a significant association between the breastfeeding intervention and metabolic syndrome possibly because of substantial overlap in breastfeeding duration and exclusivity in the two groups, which reduced heterogeneity [89].

According to the quality assessment scoring system, the overall quality of the articles was fair to moderate. Three studies were categorized as having good quality, two had moderate quality, two had fair to moderate quality, and four had fair quality. For each category, there was a large range of scores. Overall, studies scored highest in the detection of metabolic syndrome and study design, followed by the collection of breastfeeding data. Articles scored lowest in handling missing information and controlling for confounding variables. For detection of metabolic syndrome, most articles collected the anthropometric data and biomarkers needed for diagnosis, but failed to collect medical history of diseases. This could have potentially caused misclassification of subjects. Since criteria state that the presence of diabetes, lipid abnormalities, or hypertension can be substituted for biomarker levels in diagnosis, those that did not meet biomarker levels but have the disease would not be reported as having the risk factor for metabolic syndrome. Overall, the methods in diagnosing metabolic syndrome were of moderate quality, with weaknesses in reporting of assays and instruments used and also in repeat measurements of anthropometric measures. In the sampling process area, the average score was higher due to the fact that many of the articles used a random sampling scheme to select participants. This aids in the selection of a non-biased sample. However, many articles were of cross-sectional design so did poorly in the study design aspect. Many of the articles also sampled from non-generalizable sources, such as specialty clinics, which reduced external validity of the

results. For the collection of breastfeeding data, articles did moderately overall. Some articles were descriptive in their breastfeeding definition and included length and exclusively. However, many only investigated the effect of ever being breastfed. In addition, many articles collected breastfeeding data years after infancy so results were subject to recall bias. In the missing information category, the majority of articles failed to provide participation rates and information on missing data. Two out of three cohort studies provided follow-up rates. Out of these measures, articles did worst in reporting and handling of missing data. Most articles did not provide further analyses of missing information, which lowered the validity of the results. Without investigating sources of missing information, results may be subject to bias. For confounding variables, the articles did fairly in all variables categories. However, three of the articles did not include a multivariate analysis or include breastfeeding in their final model [88,93,94]. Family history and maternal factors were slightly less controlled for overall, while demographics and birth factors were better controlled for. For family history of disease, the majority of articles only controlled for one or none of the diseases of interest. Family history of diabetes and heart disease were the most commonly included confounding variables, while family history of obesity and hypertension were the least. Among maternal factors, weight or BMI, education, and income were the most controlled for, while mothers age, presence or absence of gestational diabetes, and smoking during pregnancy were least included. Strengths and limitations of the individual articles are described in more detail in Table A15 of Appendix A.

Since breastfeeding was expected to be protective for metabolic syndrome, odds ratios below one were expected. The majority of odds ratios reported by high quality articles met this expectation. Lower quality articles reported odds ratios that were inconsistent and had a large

range of values. In general, the lowest quality articles failed to find a significant inverse association between breastfeeding and metabolic syndrome, while the higher quality articles established significant associations. However, one high quality article was the exception to this trend and failed to establish a significant inverse association. This article may have failed to find a protective association due to loss of heterogeneity of the sample due to the presence of substantial overlap of the intervention arms in duration of breastfeeding, or perhaps due to the study sample itself. Sampling from a breastfeeding trial may reduce heterogeneity, since subjects may be inherently different than the general population. In addition, investigators did not measure two of the biomarkers required to diagnose metabolic syndrome, which may result in misclassification of cases [89].

B. Mechanisms

Breastfeeding may offer protection from metabolic syndrome by supplying infants with a balanced, nutrient-dense meal that contains hormones that affect food regulation. These hormones, such as leptin and ghrelin, can affect glucose-insulin metabolism and hypothalmatic development and influence weight gain [54-56]. These compounds are active in developmental programming that changes physiological processes affecting energy balance regulation [54]. In addition, breast milk prevents unhealthy weight gain in infants compared to formula due to the high energy and fat content in formula [60]. Unhealthy weight gain in infancy is related to childhood weight gain and reduced satiety [61,62], so controlling weight gain in infancy may reduce childhood obesity. Since obesity is a catalyst for metabolic syndrome due to its involvement in the development of other risk factors, reducing obesity may stem metabolic syndrome.

C. Strengths and limitations

One of the strengths of this review is that wide inclusion criteria were used, so a large array of study designs and populations were included. Additionally, the articles were assessed using a quality assessment scoring system. This allowed for the comparison of results from high and low quality studies.

A limitation of this review is that the articles were judged based on the most standard metabolic syndrome definition, International Diabetes Federation (IDF), and not all articles used this set of criteria. This could potentially cause misclassification error of an article by assigning a lower quality category solely because the study adhered to different criteria. However, the IDF criteria are internationally recognized, agreed upon by experts, and targeted for worldwide use; as such, IDF guidelines are the most appropriate guidelines to use. Another limitation is that not all studies used the appropriate ages for the metabolic syndrome criteria applied [88,90,91,93]. For instance, an article used the National Cholesterol Education Program (NCEP) adult metabolic syndrome criteria for children [93]. This may have caused misdiagnosis of metabolic syndrome. However, excluding these articles would severely limit the number of articles included in the review. Another limitation of this review is that the articles did not differentiate between bottle-feeding breast milk and actual breastfeeding. It has been demonstrated that infants have the ability to self-regulate food intake, but infants tend to consume excess calories when bottle-fed so this is an important aspect to include [100,101]. In addition, all of the categories in the quality assessment scoring system were weighted the same, even though some could argue that certain categories are more important than others, such as diagnosis of metabolic syndrome. Another limitation is that a meta-analysis was not conducted. However, there is limited research on this topic and little heterogeneity between studies, so a meta-analysis

was not conducted. There was a large amount of variation in the definitions of breastfeeding that were used (e.g. ever breastfed, exclusively breastfed for three months), age ranges of the study samples, and also locations of the studies. In the future after more studies are published, a meta-analysis would be more useful. This way the results may be calculated for specific populations and also by different definitions of breastfeeding and age ranges. In addition, no studies have been conducted in the U.S., so the results cannot yet be generalized to U.S. children and adolescents. Lastly, risk of bias within studies was examined using the quality assessment scoring system, but there is potential for risk of bias among studies. Publication bias, which may result in a larger proportion of published studies with significant findings irrespective of quality, could artificially increase the amount of evidence for a significant association. If publication bias was strong, lower quality articles that found significantly protective associations would be more frequent. However, the results indicated that the articles that were more likely to find an association were of higher quality, which provides evidence for a true association.

D. Further research

Improvements in study design, more rigorous ascertainment of exposure and outcome, and inclusion of confounding variables are needed to determine if there is truly an association between being breastfed and metabolic syndrome in children and adolescents. Longitudinal cohort studies with large sample sizes and a representative population that assess breastfeeding exclusivity, length, and bottle-feeding both breast milk and formula are necessary. Implementation of a randomized breastfeeding education trial (similar to the trial in Martin et al., but with methodological improvements in the diagnosis of metabolic syndrome) is an important addition to this topic area. Since breastfeeding itself cannot be randomized due to ethical reasons, randomizing an educational intervention that seeks to increase breastfeeding rates is a lucrative

alternative strategy. Another alternative strategy to randomization is the use of propensity scores, which is a statistical technique that accounts for other covariates that affect the probability of receiving the "treatment," which in this case is breastfeeding. Propensity scores are calculated based on the likelihood of breastfeeding and then mothers with similar likelihoods are matched. This matching process mimics randomization because it creates subunits of subjects that received the exposure and did not receive the exposure with similar covariates. In addition, future studies should use the IDF definition to increase consistency when reporting results and only include children ten years and older to be consistent with current IDF standards. Also, the inclusion of important confounders, such as demographics, maternal factors, birth factors, and disease history, is essential in future studies. Other methods to control for unmeasured confounding can also be utilized, such as investigating effects of breastfeeding among children from the same family. This will control for unmeasured confounding variables including genetics, environment, and diet. In addition, natural experiments may be conducted to look at nationwide trends in breastfeeding guidelines and policies to determine how these changes affect metabolic syndrome prevalence in different countries. This may help control for confounding variables such as culture and genetic predisposition.

Furthermore, future studies should be conducted in the U.S. Since the U.S. has lower breastfeeding rates than various other regions, such as Africa, parts of Asia, and many other developing countries [73], as well as a high prevalence of obesity, research in this location is especially pertinent.

Other important factors that could affect breastfeeding status and metabolic syndrome risk were not investigated in this review that deserve attention in future studies. An issue that was not addressed in the articles was child lifestyle factors, including diet and exercise, which

can affect risk of metabolic syndrome. A child's poor diet in early childhood may reduce the potentially protective effect of being breastfed. Even though diet and exercise may be difficult to measure, there are many standardized questionnaires that can be utilized to capture this information. Another potential confounding variable that should be addressed in future studies may be insulin resistance in the mother. Insulin has been implicated as playing a direct role in breastfeeding, and insulin resistance may hinder successful lactation [102]. A closer look into what effect modifiers may be altering the association between being breastfed and metabolic syndrome, including gender, age, or puberty status, is also needed. For instance, the protective effect of being breastfed on metabolic syndrome may only be present early in childhood, and more so among females. The articles failed to investigate if the effect of being breastfed is different in subgroups of the population.

E. Conclusions

This review helps to fill current gaps in understanding on the relationship between breastfeeding and metabolic syndrome development in children and adolescents. In public health, it is necessary to summarize current research to assess what further research steps are necessary and to determine if there is enough evidence to implement public health interventions. Based on the findings from this review, it is apparent that evidence suggests that there is a protective relationship between breastfeeding and metabolic syndrome in children and adolescents. However, more research is needed to clarify this relationship. For future studies, methodological improvements are necessary in study design, the handling missing information, detection of metabolic syndrome, collection of breastfeeding data, and control of confounding variables. If a relationship is established, interventions can be put in place to encourage breastfeeding for those populations most at risk for metabolic syndrome. In addition, more

information on the proper length and exclusivity of breastfeeding in relation to metabolic syndrome can tailor interventions for those most at risk, including low birth weight and pre-term babies. Implementing interventions to reduce metabolic syndrome may be important because it is a risk factor for cardiovascular disease and diabetes.

APPENDICES

APPENDIX A: Additional tables
Table A1: Criteria for diagnosing metabolic syndrome

Criteria by Organization or Author	
De Ferranti et al 2004 [103]	
Children (12-19 years old)	Extrapolated the adult NCEP ATP III criteria. Any three of the following are required for diagnosis of MetS: - Hypertriglyceridemia: ≥1.1 mmol/L - Low HDL: HDL <1.3 mmol/L (boys aged 15-19, <1.17 mmol/L) - High fasting glucose: ≥6.1 mmol/L - Central obesity (waist circumference): >75th percentile for age and gender - Hypertension: >90th percentile for age, gender, and height
European Group for the Study of Insulin Resistance (EGIR) [104]	
All ages	For non-diabetic individuals only. Defined by presence of insulin resistance of fasting hyperinsulinemia (the highest 25%) and two of the following: - Hyperglycemia (fasting plasma glucose ≥6.1 mmol/l, but non-diabetic) - Hypertension (systolic/diastolic blood pressure ≥140/90 mmHg or treated for hypertension) - Dyslipidemia (triglycerides >2.0 mmol/l or HDL-cholesterol <1.0 mmol/l or treated for dyslipidemia) - Central obesity (waist circumference ≥94 cm in men and ≥80 cm in women)
International Diabetes Federation (IDF) [39]	
Children: 6-<10 years	Metabolic syndrome cannot be diagnosed, but further measurements should be made if family history of metabolic syndrome, type 2 diabetes mellitus, dyslipidemia, cardiovascular disease, hypertension, or obesity
Children and adolescents: 10- <16 years	- Obesity >90th percentile (or adult cut-off if lower) as assessed by waist circumference - Triglycerides >1.7 mmol/L - HDL-cholesterol <1.03 mmol/L - Blood pressure >130 mm Hg systolic or >85 mm Hg diastolic - Glucose >5.6 mmol/L (oral glucose tolerance test recommended) or known type 2 diabetes mellitus
Adults: 16 years and older	Central obesity (defined as waist circumference* with ethnicity specific values) plus any two of the following four factors: - Raised triglycerides: ≥150 mg/dL (1.7 mmol/L) or specific treatment for this lipid abnormality - Reduced HDL cholesterol: <40 mg/dL (1.03 mmol/L) in males and <50 mg/dL (1.29 mmol/L) in females or specific treatment for this lipid abnormality - Raised blood pressure: systolic BP ≥130 or diastolic BP ≥85 mm Hg or treatment of previously diagnosed hypertension - Raised fasting plasma glucose: ≥100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes. If above 5.6 mmol/L or 100 mg/dL, OGTT is strongly recommended but is not necessary to define presence of syndrome * If BMI >30 kg/m^2, central obesity can be assumed and waist circumference does not need to be measured.

Table 1 (con't)

National Cholesterol Education Program Adult Treatment Panel (NCEP ATP) III [104]	
Adults: 20 years and older	Clinical identification of the metabolic syndrome requires any three of the following: - Abdominal obesity*: Men >102 cm (>40 in), Women >88 cm (>35 in) - Triglycerides: ≥150 mg/dL - HDL cholesterol: Men <40 mg/dL, Women <50 mg/dL - Blood pressure: ≥130/≥85 mmHg - Fasting glucose: ≥110 mg/dL * Waist circumference is recommended to measure abdominal obesity, not BMI
Weiss et al 2004 [105]	
Children and adolescents	Modified from the NCEP ATP III and WHO criteria. Diagnosed as metabolic syndrome if three or more of the following criteria for age and sex are present: - BMI above the 97th percentile (z score, 2.0 or more) - Triglyceride level above the 95th percentile - HDL cholesterol level below the 5th percentile - Systolic or diastolic blood pressure above the 95th percentile - Impaired glucose tolerance: >140 mg per deciliter (7.8 mmol per liter) but less than 200 mg per deciliter (11.1 mmol per liter) at two hours
World Health Organization (WHO) [104]	
All ages	Glucose intolerance, impaired glucose tolerance, or diabetes mellitus and/or insulin resistance together with two or more of these components: - Impaired glucose regulation or diabetes - Insulin resistance (under hyperinsulinaemic euglycaemic conditions, glucose uptake below lowest quartile for background population under investigation) - Raised arterial pressure ≥160/90 mmHg - Raised plasma triglycerides (≥1.7 mmol 1^-1; 150 mg dl^-1) and/or low HDL-cholesterol (<0.9 mmol l^-1, 35 mg dl^-1 men; <1.0 mmol l^-1, 39 mg dl^-1 women) - Central obesity (males: waist to hip ratio >0.90; females: waist to hip ratio >0.85) and/or BMI >30 kg m^-2 - Micro albuminuria (urinary albumin excretion rate ≥20 μg min^-1 or albumin:creatinine ratio ≥20 mg g^-1) Several other components of the metabolic syndrome have been described (e.g. hyperuricaemia, coagulation disorders, raised PAI-1) but they are not necessary for the recognition of the condition.

Table A2: Search results

Order of search	Source	Search terms	Total results	Fit criteria*	Secondary search through reference lists	Total
1	Pub Med	"Breastfeeding" and "Metabolic syndrome in children"	118	6	1	7
2	Pub Med	"Infant feeding" and "Metabolic Syndrome"	51	1	1	2
3	Pub Med	"Infant formula" and "Metabolic Syndrome"	44	0	0	0
4	Embase	"Breastfeeding" and "Metabolic syndrome in children"	107	1	1	2
5	Embase	"Infant feeding" and "Metabolic Syndrome"	437	0	0	0
6	Embase	"Infant formula" and "Metabolic Syndrome"	72	0	0	0
Total			**829**	**8**	**3**	**11**

***Plus has not already been selected by previous searches**
Search conducted 12/29/2015

Table A3: General description of studies

First author (year)	Study design	Population or dataset	Location	Metabolic syndrome guidelines
Ekelund (2009)	Cross-sectional	Sampled from the European Youth Heart Study (EYHS): a multicenter mixed longitudinal study	Estonia, Denmark, and Portugal	IDF criteria (used age-specific reference data from randomly selected British children when defining WC cut-off points)
Esfarjani (2013)	Cross-sectional	Subjects from a field trial of a family-based intervention for controlling childhood obesity	Tehran, Iran	NCEP ATP III (modified for pediatric population, WC and BP percentiles determined according to national references curve)
Folic (2015)	Case-control	Patients treated on an inpatient basis at the Endocrine Department of the Pediatric Clinic at the Clinical Centre Kragujevac, Kragujevac, Serbia	Kragujevac, Serbia	IDF criteria (WC cut-off points according to Yugoslav standards)
González-Jiménez (2015)	Cross-sectional	Subjects from the sixth grade of primary school to the third year of secondary school from 18 schools	Granada and Almeria provinces in Spain	IDF criteria for 10-16 year olds
Jamoussi (2012)	Cross-sectional	Subjects recruited from the research unit of the National Institute of Nutrition	Tunis, Tunisia	IDF criteria (for those <16, used the criteria for 10-16 year olds, for those 16 years and older, used recommended WC cut-off points for European and sub-Saharan African populations)
Khuc (2012)	Retrospective Cohort	Subjects from a randomized controlled trial of iron supplementation to prevent iron deficiency anemia	Santiago, Chile	IDF criteria
Martin (2014)	Retrospective Cohort	Subjects followed up from the Promotion of Breastfeeding Intervention Trial, a cluster-randomized trial of a breastfeeding promotion intervention	Belarus	EGIR definition
Sen (2007)	Cross-sectional	Subjects were referred to the Division of Pediatric Endocrinology, Hacettepe University Children's Hospital	Ankara, Turkey	WHO criteria and NCEP ATP III guidelines
Wang, J (2015)	Cross-sectional	Ten schools selected in an urban area of a large and wealthy city in South China	Guangzhou, China	IDF criteria adapted for children and adolescents (WC cut-off points for Chinese children used)
Wang, S (2015)	Cross-sectional	Students randomly selected from urban and rural areas	Wuhan, China	De Ferranti et al. criteria (sex and age specific cutoff points for Chinese children) and IDF criteria
Yakubov (2015)	Retrospective Cohort	Subjects were enrolled that visited the pediatric or pediatric nephrology outpatient clinics of the Hillel Yaffe Medical Center for hypertension and/or obesity	Harera, Israel	Weiss et al. definition

Table A4: Aspects of the sample

First author (year)	Sampling process	Age at outcome in years (mean or median if available)	Sample size (cases, controls)	Completeness of data
Ekelund (2009)	- Minimum of 20 schools at each study location randomly selected with appropriate age, sex, and socioeconomic strata - Children randomly selected within schools	10 and 15	3193 (27, 3166)	73% response rate
Esfarjani (2013)	-Obese children (BMI ≥95th percentile) randomly selected from 9 health centers in three districts of north Tehran - Exclusion criteria: genetic syndromes, any chronic disease or disability, history of chronic medication use, special diet	7 (mean 6.65)	150 (20, 130)	Not reported
Folic (2015)	- From 2008 to 2012, children and adolescents with obesity/metabolic syndrome that were treated on an inpatient basis at the Pediatric Clinic of the Clinical Centre - Controls randomly selected from the source population and matched to cases by gender, age, and comorbidity (allergic rhinitis, asthma, or epilepsy) - Controls in the 90th percentile or greater for waist circumference	10-16 (mean 12.93 in cases, 12.43 in controls)	84 (28, 56)	Not reported
González-Jiménez (2015)	Sixth grade of primary school to third year of secondary school	10-15	976 (43, 933)	Not reported
Jamoussi (2012)	- Overweight and obese children and adolescents recruited in the research unit on human obesity of the National Institute of Nutrition from 2007 to 2008 - Exclusion criteria: obesity secondary to endocrinological and genetic diseases and pharmacological agents, and those on medication	6-18 (mean 13.50)	186 (64, 122)	Not reported
Khuc (2012)	- Adolescents enrolled in a randomized controlled trial of iron supplementation to prevent iron deficiency anemia randomly selected - Original enrollment 1991-1996 - Inclusion criteria for the trial: birth weight three kg or more, no birth complications, major congenital abnormalities, or iron therapy	16-17 (mean 16.6 in males, mean 16.7 in females)	357 (37, 320)	93% of selected subjects had complete data

Table A4 (con't)

First author (year)	Sampling process	Age at outcome in years (mean or median if available)	Sample size (cases, controls)	Completeness of data
Martin (2014)	- Children in the Promotion of Breastfeeding Intervention Trial from 2008 to 2010 - Cluster-based randomization used in the original trial - Units of randomization: maternity hospitals and associated polyclinics - Units randomized to a control group or experimental intervention to promote and support breastfeeding - Trial inclusion: healthy baby, singleton, birth weight of 2500 grams or more, APGAR score of five or greater at five minutes - Mothers excluded if they had a condition that interfered with breastfeeding	11.5	13616 (Observational portion: 475, 13141)	84.5% of children were successfully followed up from the original trial, 97% participated that had been successfully followed-up
Sen (2007)	- Obese children (BMI ≥ 95th percentile) referred to the Division of Pediatric Endocrinology, Hacettepe University Children's Hospital between March 2003 and March 2005 - Exclusion criteria: obesity secondary to endocrinolgical, genetic diseases, and pharmacological, and those on medication	2-19 (median 11.8)	352 (147, 205)	Not reported
Wang, J (2015)	- First stage: four districts from the urban area randomly selected - Second stage, ten schools selected in the four districts - Third stage: two classes per grade randomly selected and invited to participate - Exclusion criteria: Children with missing information such as age, sex, or BMI were excluded, those with serious health problems - All participants required to be ethnic Han	7-17 (mean 11.3)	1770 (19, 1751)	87% response rate, 97% of subjects had complete data
Wang, S (2015)	- Four districts randomly selected (2 urban and 2 suburban) - 1 primary school randomly selected in each district - Students in grades 3 and 4 invited to participate	Unavailable (mean 9.6)	624 (42, 582)	Not reported
Yakubov (2015)	- Obese children aged 3-18 years who visited the pediatric or pediatric nephrology outpatient clinics of a single center for hypertension and/or obesity in 2008-2012 - Exclusion criteria: insufficient laboratory data, presence of a disease and/or a medicinal treatment that could affect metabolic functions or patient's weight	3-18	123 (58, 65)	73% of those had complete information

Table A5: Results

First author (year)	Test	Result
Ekelund (2009)	Chi-square test to determine if prevalence of breastfeeding different between those with MetS and those without	p-value= 0.002
Esfarjani (2013)	Chi-square test and t-test to determine if means and ratios different of breastfeeding 6 or more months between those with MetS and those without	p-value= 0.045
	Multivariate logisic regression to determine if breastfeeding 6 or more months if significantly related to MetS	Not reported, but not significant
	Chi-square test and t-test to determine if means and ratios different of ever breastfeeding between those with MetS and those without	p-value not reported, but not significant
	Multivariate logistic regression to determine if ever breastfeeding if related to the MetS	Not reported, but not significant
Folic (2015)	Pearson's Chi-square test to determine if breastfeeding during first 6 months of life is different in those with MetS compared with the obese group without MetS	p-value= 0.014
	Multivariate logistic regression to determine if breastfeeding during the first 6 months of life is significantly associated with MetS	OR= 0.079, 95% CI (0.009-0.716); p-value= 0.024
González-Jiménez (2015)	Logistic regression to determine if length of breastfeeding is associated with MetS	OR(1-3 months)= 3.26, 95% CI (0.92-10.85); OR(4-6 months)= 1.70, 95% CI (0.39-7.45); OR(>6 months)= 0.13, 95% CI (0.03-0.65)
Jamoussi (2012)	Univariate analysis to determine if ever breastfeeding was different in MetS and non-MetS group	70.3% of those with MetS were breastfed and 64.80% of those without MetS were breastfed, p-value not reported, but not significant
Khuc (2012)	Linear regression to determine if introduction of the first bottle at 90 or after is related to MetS	Coefficent=-0.16, p-value <0.05
Martin (2014)	Logistic regression to determine if diagnosis of MetS is different in intervention vs control arm of the breastfeeding trial using intention-to-treat analysis	OR (cluster adjusted)= 1.21, 95% CI (0.85-1.72); OR (further adjusted for baseline factors)= 1.16, 95% CI (0.81-1.66)
	Logistic regression to determine if diagnosis of MetS is different by length of breastfeeding (<3 months, 3-<6 months, 6 or more months), but using randomized treatment as an instrumental variable	Cluster adjusted: OR(<3 months)= 1.0(ref); OR(3-<6 months)= 1.84, 95% CI (0.66-5.15); OR(6 or more months)= 2.32, 95% CI (0.47-11.43) Further adjusted for baseline factors: OR(<3 months)= 1.0(ref); OR(3-<6 months)= 1.91, 95% CI (0.72-5.05); OR(6 or more months)= 2.23, 95% CI (0.52-9.68)
	Logistic regression to determine if diagnosis of MetS is different in by length of breastfeeding (<3 months, 3-<6 months, 6 or more months)	Cluster adjusted: OR(<3 months)= 0(ref); OR(3-<6 months)= 1.08, 95% CI (0.85-1.37); OR(6 or more months)= 1.09, 95% CI (0.65-1.81); Trend p-value= 0.54 Further adjusted for baseline factors: OR(<3 months)= 0(ref); OR(3-<6 months)= 1.09, 95% CI (0.86-1.39); OR(6 or more months)= 1.14, 95% CI (0.68-1.89); Trend p-value= 0.43

Table A5 (con't)

First author (year)	Test	Result
Sen (2007)	Chi-square test and Student's t-test to compare average duration of breastfeeding in MetS group compared to the non-MetS group	Those with MetS had a median length of breastfeeding of 10 months with a range of 0-36 months, those without MetS had a median length of breastfeeding of 9 months with a range of 0-24 months: no value reported, but not significantly different
Wang, J (2015)	Multiple logistic regression to determine if length of breastfeeding is associated with MetS (<1 month, 1-3 months, 4-6 months, >6 months)	OR(<1 month)= 1(Ref); OR(1-3 months)= 0.57, 95% CI (0.19-1.75); OR(4-6)= 0.79, 95% CI (0.35-1.78); OR(>6 months)= 0.39, 95% CI (0.16-0.98)
Wang, S (2015)	Chi-square test and t-test to determine if ever breastfeeding is different in those with MetS and those without	p-value= 0.826
	Multivariate analysis to determine if ever breastfeeding is associated with MetS	OR= 0.32, 95% CI (0.10-0.97); p-value= 0.044
Yakubov (2015)	Chi-square test was ran separately in MetS subjects and Non-MetS subjects to determine if there are different prevalences of breastfeeding subgroups (0-2 months, 2-6 months, 6 months or more) within the two groups	Metabolic syndrome: p-value= 0.99; Non-metabolic syndrome: p-value= 0.98

Table A6: Category 1 quality assessment scores

Category 1: Sampling Process				
First author (year)	**Study design**	**Sample selection**	**Population source**	**Average for Category 1**
Ekelund (2009)	1	5	5	3.7
Esfarjani (2013)	1	5	2	2.7
Folic (2015)	1	5	2	2.7
González-Jiménez (2015)	1	1	5	2.3
Jamoussi (2012)	1	1	1	1.0
Khuc (2012)	5	5	3	4.3
Martin (2014)	5	5	3	4.3
Sen (2007)	1	1	2	1.3
Wang, J (2015)	1	5	4	3.3
Wang, S (2015)	1	5	5	3.7
Yakubov (2015)	5	1	1	2.3
Average	**2.1**	**3.5**	**3**	**2.9**

1= Poor
2= Fair
3= Moderate
4= Good
5= Excellent

Table A7: Category 2 quality assessment scores

Category 2: Missing Information				
First author (year)	**Participation rate or response rate**	**Loss to follow-up reported: cohort studies only**	**Missing data**	**Average for Category 2**
Ekelund (2009)	3	N/A	1	2
Esfarjani (2013)	1	N/A	1	1
Folic (2015)	1	N/A	1	1
González-Jiménez (2015)	1	N/A	1	1
Jamoussi (2012)	1	N/A	1	1
Khuc (2012)	1	5	4	3.3
Martin (2014)	5	5	1	3.7
Sen (2007)	1	N/A	1	1
Wang, J (2015)	4	N/A	5	4.5
Wang, S (2015)	1	N/A	1	1
Yakubov (2015)	1	1	2	1.3
Average	**1.8**	**3.7**	**1.7**	**1.9**

1= Poor
2= Fair
3= Moderate
4= Good
5= Excellent

Table A8: Category 3 quality assessment scores

Category 3: Detection of metabolic syndrome			
First author (year)	**Criteria included**	**Quality of methods**	**Average for Category 3**
Ekelund (2009)	5	5	5
Esfarjani (2013)	4	4	4
Folic (2015)	5	3	4
González-Jiménez (2015)	4	3	3.5
Jamoussi (2012)	4	4	4
Khuc (2012)	4	2	3
Martin (2014)	3	3	3
Sen (2007)	5	3	4
Wang, J (2015)	4	5	4.5
Wang, S (2015)	4	3	3.5
Yakubov (2015)	4	1	2.5
Average	**4.2**	**3.3**	**3.7**

1= Poor
2= Fair
3= Moderate
4= Good
5= Excellent

Table A9: Category 4 quality assessment scores

Category 4: Breastfeeding data			
First author (year)	**Definition of breastfeeding**	**Breastfeeding data source**	**Average for Category 4**
Ekelund (2009)	1	1	1
Esfarjani (2013)	3	1	2
Folic (2015)	2	1	1.5
González-Jiménez (2015)	4	3	3
Jamoussi (2012)	1	1	1
Khuc (2012)	5	5	5
Martin (2014)	5	5	5
Sen (2007)	4	1	2.5
Wang, J (2015)	4	1	2.5
Wang, S (2015)	1	1	1
Yakubov (2015)	4	5	4.5
Average	**3.1**	**2.3**	**2.6**

1= Poor
2= Fair
3= Moderate
4= Good
5= Excellent

Table A10: Category 5 quality assessment scores

Category 5: Control of confounding					
First author (year)	**Demographics**	**Maternal factors**	**Family history**	**Birth factors**	**Average for Category 5**
Ekelund (2009)	3	2	1	1	1.8
Esfarjani (2013)	3	4	3	5	3.8
Folic (2015)	1	4	5	1	2.8
González-Jiménez (2015)	3	3	1	3	2.5
Jamoussi (2012)	1	1	1	1	1
Khuc (2012)	3	1	5	1	2.5
Martin (2014)	3	2	1	3	2.3
Sen (2007)	1	1	1	1	1
Wang, J (2015)	5	2	1	3	2.8
Wang, S (2015)	3	2	3	5	3.3
Yakubov (2015)	1	1	1	1	1
Average	**2.5**	**2.1**	**2.1**	**2.3**	**2.3**

1= Poor
2= Fair
3= Moderate
4= Good
5= Excellent

Table A11: Detection of metabolic syndrome (anthropometric measures and blood pressure)

First author (year)	Body mass index	Waist circumference	Blood pressure
Ekelund (2009)	- Height and weight measured using standard techniques - Participants wore light clothing and no shoes	- Measured two times with a metal anthropometric tape midway between the lower rib margin and the iliac crest at the end of a gentle expiration - Average of two measurements	- Measured after the subject rested for five minutes sitting down with a Dinampa vital-signs monitor - Means of the last three measurements
Esfarjani (2013)	- Weight measured to nearest 0.1 kg using a calibrated and certified portable digital scale with light clothes, no shoes, and empty pockets - Height measured in a standing position, barefoot, and using a portable height gauge with accuracy of 0.1 cm - Mean of two measurements	- Measured with accuracy of 0.1 cm at smallest area between the edge of the lower crest and the iliac crest bone	- Measured using manual standard mercury sphygmomanometer from the right arm after 5-10 minutes of rest - Average of two measurements at the observer's eye level
Folic (2015)	- Height and weight measured without the subjects wearing shoes or heavy clothing - Height measured to nearest 0.1 cm and weight to nearest 0.1 kg.	- Measured at the level of the narrowest point between the lower costal border and the illiac crest with non-stretchable measuring tape while patient exhaled	- Patients relaxed for more than 10 minutes - Measured two times five minutes apart
González-Jiménez (2015)	- Weighed on self-calibrating digital floor scale with precision of up to 100 g - Height measured using portable stadiometer - Subject stood erect with back, buttocks, and heels in contact with the height rod and head oriented in the Frankfurt plane	- Measured using horizontal plane midway between the lowest rib and the upper border of the iliac crest at the end of normal expiration - Automatic roll-up measuring tape used	- Calculated with aneroid sphygmomanometer and stethoscope - Subjects sat down and relaxed for 10 minutes and blood taken from the right arm - Results compared with international reference standards
Jamoussi (2012)	- Weight measured with portable scale and height with wall-mounted stadiometer	- Measured at midpoint between lowest rib and iliac crest along the mid-axillary line at end of expiration	- Measured using mercury sphygmomanometer after five minutes of rest
Khuc (2012)	- Weight measured to nearest 0.1 kg using a SECA scale - Height measured to the closest 0.1 cm using a Holtain stadiometer - Measurements taken twice, with an additional third measurement if the difference between the first two was greater than 0.3 kg for weight and 0.5 cm for height	- Method not reported	- Method not reported
Martin (2014)	- Not measured	- Measured in duplicate	- Measured in triplicate
Sen (2007)	- Method not mentioned, but measured by same person.	- Not measured	- Measured by manometer with cuff on the right arm after five minutes of rest - Values compared with standard percentiles for age and gender

Table A11 (con't)

First author (year)	Body mass index	Waist circumference	Blood pressure
Wang, J (2015)	- Fasting body weight measured to the nearest 0.1 kg on double ruler scale - Height measured to accuracy of 1 mm with a free-standing stadiometer mounted on a rigid tripod - Subjects stood erect with back, buttocks, and heels in contact with the vertical height rod of the stadiometer and head oriented in the Frankfurt plane - Horizontal headpiece was placed on top of the head of the subject	- Measured using the horizontal plane midway between the lowest rid and the upper border of the iliac crest at the end of a normal inhale and exhale - Average of two consecutive measures	- Using a mercury sphygmomanometer after a fifteen minute rest - Tested twice on the right arm, average of two readings obtained at a minimum of five minutes apart
Wang, S (2015)	- Weight measured in light clothes - Height measured standing erect without shoes	- Method not mentioned, measured using standard methods	-Measured sitting in an upright position for at least five minutes -Two measurements taken in the morning, mean recorded
Yakubov (2015)	- Height and weight measured using standard procedures	- Not measured	- Measured using the oscillometric method

Table A12: Detection of metabolic syndrome (biochemistry and disease history)

First author (year)	Triglycerides	HDL-C	Plasma glucose	Insulin	Disease history
Ekelund (2009)	- Measured after an overnight fast - Samples from Denmark and Estonia measured in one laboratory, samples for Portugal tested in another laboratory - Measured by enzymatic methods	- Measured after an overnight fast - Samples from Denmark and Estonia measured in one laboratory, samples for Portugal tested in another laboratory - Measured by enzymatic methods	- Measured after an overnight fast - Samples from Denmark and Estonia measured in one laboratory, samples for Portugal tested in another laboratory - Measured by hexokinase method and measured with an Olympus autoanalyzer	- Measured after an overnight fat with an enzyme immunoassay	- Diagnosis of diabetes was collected
Esfarjani (2013)	- After 12-hour overnight fast, measured using a commercial kit based on the enzymatic methods with auto-analyzer	- After 12-hour overnight fast, measured using a commercial kit based on the enzymatic methods with auto-analyzer	- After 12-hour overnight fast, measured using a commercial kit based on the enzymatic methods with auto-analyzer	- Not measured	- Not collected
Folic (2015)	- Performed in the Laboratory Diagnostics Department according to standard operating procedures	- Performed in the Laboratory Diagnostics Department according to standard operating procedures	- Performed in the Laboratory Diagnostics Department according to standard operating procedures	- Measured fasting and at 120 minutes using the OGTT - Measured insulin sensitivity with HOMA and QUICKI tests	- Diagnosis of diabetes was collected
González-Jiménez (2015)	- Frozen after collection at 8:00 am after a 12-hour overnight fast - Measured by the enzymatic colorimetric method with an Olympus analyzer	- Frozen after collection at 8:00 am after a 12-hour overnight fast - Measured by the enzymatic colorimetric method with an Olympus analyzer	- Measured after collection at 8:00 am after a 12-hour overnight fast - Measured by using the colorimetric enzymatic method	- Plasma insulin measured after overnight fast using an ELISA kit	- Not collected
Jamoussi (2012)	- After overnight fast, measured by enzymatic methods	After overnight fast, measured by enzymatic methods	After overnight fast, measured by enzymatic methods	Not measured	- Not collected

Table A12 (con't)

First author (year)	Triglycerides	HDL-C	Plasma glucose	Insulin	Disease history
Khuc (2012)	- Collected fasting - Enzymatic-colorimetric test	- Collected fasting - Enzymatic-colorimetric test	- Collected fasting - Enzymatic-colorimetric test	- Not measured	- Not collected
Martin (2014)	- Not measured	- Not measured	- Fasting blood test	- Measured circulating insulin from two dried blood spot samples that are based on an adaptation of an existing commercial kit	- Hypertension and diabetes reported
Sen (2007)	- Measured using a modular analytical system in fasting blood samples	- Measured in fasting blood samples - Modular analytical system	- Glucose oxidase method used to measure glucose levels 0, 30, 60, 90, and 120 minutes post-ingestion - OGTT performed after a 12-hour fast	- Measured in blood samples at 0, 30, 60, 90, and 120 minutes post-ingestion using radioimmunoassay - Insulin resistance analyzed using HOMA and OGTT	- History of diabetes was collected
Wang, J (2015)	- After 12-hour overnight fast, assayed using enzymatic methods	- After 12-hour overnight fast, calculated using the clearance method	- After 12-hour fast, measured using the glucose oxidase method	- Not collected	- Not collected
Wang, S (2015)	-Fasting blood test	- Fasting blood test	- Fasting blood test	- Not measured	- Not collected
Yakubov (2015)	- Performed in the hospital laboratory after 12-hour fast sample taken	- Performed in the hospital laboratory after 12-hour fast sample taken	- Performed in the hospital laboratory after 12-hour fast sample taken	- Insulin blood levels measured after 12-hour fast	- Not collected

Table A13: Breastfeeding data

First author (year)	Definition of exposure: breastfeeding	Detection of exposure: breastfeeding
Ekelund (2009)	Ever breastfed	Both parents completed a questionnaire separately
Esfarjani (2013)	Ever breastfed, and also less than 6 months or 6 months and greater	Parents completed a questionnaire through an interview
Folic (2015)	Ever breastfed during first 6 months of life	Interview with parents
González-Jiménez (2015)	Duration in months (categorical)	Extracted from histories of each mother, and verified by a questionnaire
Jamoussi (2012)	Ever breastfed	Recorded from interviews with the parents
Khuc (2012)	Breastfeeding as sole source of milk for 90 or more days	Date of first supplemental bottle reported by mother during monthly exams
Martin (2014)	Intervention arm: intervention based training personnel on methods to maintain lactation, promote exclusive and prolonged breastfeeding, and to resolve common problems Duration of exclusive breastfeeding by month (categorical) using WHO definition in observational portion of the study	Followed up women at 1, 2, 3, 6, 9, and 12 months with an interview. Polyclinic visit forms verified with polyclinic charts
Sen (2007)	Duration in months	Parents completed a self-reporting questionnaire
Wang, J (2015)	Duration in months and also exclusively breastfed 30 or more days	Questionnaire filled out by parents during an in-person interview
Wang, S (2015)	Ever breastfed	Parents completed a written questionnaire
Yakubov (2015)	Duration in months (categorical)	Not mentioned

Table A14: Covariates

First author (year)	Covariates collected	Covariates that were adjusted for or matched on
Ekelund (2009)	- Skin folds used to measure adiposy, maternal socioeconomic status, BMI, diabetes, and HT, birth weight, sexual maturity, cardiorespiratory fitness tested by an ergometer, self-reported sports participation, physical activity measured by an MTI Actigraph, mode of transportation to school, participation in clubs, smoking status, television viewing, and regular play, sexual maturation, insulin	- Age group, sex, study location, and maternal BMI - Adjusted for socioeconomic status, but it did not affect results so it was removed
Esfarjani (2013)	- Socio-demographic questionnaire that included age, birth order, birth weight and height, mothers' age, marital status, parents' occupation and education level, number of household members	- Sex, birth order, birth weight, number of household members, age of mother, gestational diabetes, parents occupation and education, and family history of obesity
Folic (2015)	- Age, gender, parental BMI, mother's pregnancy (controlled, special-care, use of medications in pregnancy, smoking, specific diseases such as gestational diabetes, pregnancy-induced hypertension, co-morbidities (parents and immediate family), socioeconomic living conditions, level of parental education, birth order, family diet (type of food, regularity of meals), smoking, physical activity, family stress events, family attitude towards obesity, APGAR score, child diet, child physical activity, child comorbidity, co-medication in children, child diet, HOMA index, fasting blood insulin and after 120 minutes, QUICKI index, uric acid, presence/absence of *Acanthosis nigricans,* heart rate, AST, ALT, GGT, HbA1c, TC, LDL-C, cortisol, microalbuminuria, creatine	- Microalbuminuria, gestational diabetes, mother's BMI and weight, father's BMI, child/adolescent cortisol levels, levels of ALT, GGT, uric acid, LDL, family history of elevated cholesterol/triglyceride levels, family history of type 2 diabetes mellitus, special-care pregnancy, maternal smoking during pregnancy, and family history of stressogenic event
González-Jiménez (2015)	- Gender, hip circumference, waist-to-hip ratio, body fat calculated from skin folds, birth weight of the student, maternal lactation period, maternal consumption of cigarettes during pregnancy, and maternal weight status during pregnancy, plasma insulin, HBA1c, LDL-C, ceruloplasmin, homocysteine, NEFA	- Gender, birth weight, cigarette consumption, and maternal weight
Jamoussi (2012)	- Age, gender, pubertal development (Tanner staging), family history of obesity and diabetes, birth weight, obesity duration (years), age at onset of obesity	- None, no multivariate analysis performed
Khuc (2012)	- Age, gender, birth weight, weight at three months, maternal education, maternal pre-pregnancy BMI calculated from height and self-report of weight, family history of type 2 diabetes, hypertension, dyslipidemia, and heart attack before the age of 60 in first degree relatives, ever iron deficiency, iron supplemented in infancy	- Weight gain in first three months and gender included in final model - Birth weight, maternal education, mother's age, maternal pre-pregnancy BMI, family history of type 2 diabetes, dyslipidemia, and heart attack, ever iron deficiency in infancy, and iron supplementation in infancy not in final model after determining they were insignificant

Table A14 (con't)

First author (year)	Covariates collected	Covariates that were adjusted for or matched on
Martin (2014)	- Gender, pubertal stage (Tanner), maternal history of hypertension, type 2 diabetes, or gestational diabetes, maternal age, maternal education, paternal education, stratum-level variable (east/urban, east/rural, west/urban, or west/rural), number of older siblings, maternal smoking during pregnancy, birth weight, paternal height and BMI, maternal height, BMI, and blood pressure, fasting insulin, adiponectin, apolipoprotein A1	- Hospitals/clinics, urban/rural, East/West, age, sex, birth weight, maternal and paternal education, and mean insulin
Sen (2007)	- Level of physical activity, birth weight, pattern of nutrition, length of formula feeding, duration of obesity, age at onset of obesity, family history of obesity, DM, hypertension, cerebrovascular events, coronary artery disease, DL, gestational diabetes in first and second degree relatives, educational status of the parents, insulin to measure insulin resistance and HI, TC, LDL-C, VLDL-C, pubertal development (Tanner staging)	- None, breastfeeding was not included in multivariate logistic regression analysis
Wang, J (2015)	- Age, child birth weight, parental education levels, family history of chronic diseases, hip circumference, waist-to-hip ratio calculated, LDL-C, passive smoking	- Birth weight, father's education, mother's education, age, and sex
Wang, S (2015)	- Age, gender, CRF, household income, parental HT, children's birth weight, preterm birth	- Cardiorespiratory fitness, birth weight, preterm birth, household income, paternal hypertension, maternal hypertension, district, sex, and age
Yakubov (2015)	- TC, LDL-C, insulin, HbA1c levels	- None

Table A15: Strengths and limitations

First author (year)	Strengths	Limitations
Ekelund (2009)	- Children from three distinct geographic locations - Maximal exercise test - Large number of covariates - Large sample size - Sampling scheme insured selection of appropriate age, sex, and socioeconomic levels - Both parents completed questionnaires	- Unmeasured confounders (e.g. dietary intake and genotype) - Birth weight and maternal BMI self-reported - Breast feeding reported retrospectively
Esfarjani (2013)	- Subjects from a variety of locations, so this will increase generalizability - Face-to-face interviews will decrease measurement error	- Small sample size - Breast feeding data collected retrospectively, so could be potential for recall bias - Exclusion criteria excludes children that take medication chronically as well as those on a special diet, so results will be less generalizable
Folic (2015)	- Many covariates taken account for, including family history of disease, diet and exercise - Inclusion of many covariates led to discovery that microalbuminuria is associated with metabolic syndrome - Matched cases and controls to common comorbidity - Cases and controls were treated the same way and underwent the same tests	- Small sample size - Only one study site - Self-report of many variables, including maternal BMI - Results may be not generalizable, since the patients were recruited to a clinic for treatment for another comorbidity - Cases and controls still very different in respect to confounders, such as BMI, waist circumference, and blood pressure - Breastfeeding data collected retrospectively
González-Jiménez (2015)	- Acceptable sample size - Large amount of schools, more diverse population - Clinical histories used to increase accuracy of measurement of breastfeeding - Included children of all BMI percentiles, so results are more generalizable to non-obese children - Questionnaire was validated with medical records	- Not prospective - No mention of the selection process of subjects - Did not record family history of diseases
Jamoussi (2012)	- Interviewing parents can reduce measurement bias, because it allows time for questions - Included age at onset of obesity, pubertal development, and family history of relevant diseases as covariates	- Recruited from the research unit on obesity, so sample may be biased - Small sample size - Not prospective - Breastfeeding measurement subject to recall bias - Used IDF criteria for children less than 10. IDF states that those less than 10 cannot be diagnosed
Khuc (2012)	- Detailed and reliable anthropometric information collected at the research center - Prospective data collection - Breastfeeding data collected from 4 to 12 months - Adolescent data collection included family history of many important diseases - Multiple follow-up points	- Enrolled from low- to middle-income community during a period of economic and nutritional transition, so may not be generalizable - Children with birth weights lower than three kg were excluded, so this limits generalizability - Formula use assessed, but information on other complementary food not assessed - Methods not mentioned for measuring waist circumference and blood pressure, which are both used to diagnose metabolic syndrome

Table A15 (con't)

First author (year)	Strengths	Limitations
Martin (2014)	- Intention-to-treat analysis - Assessed infant feeding regularly during the first year of life and used WHO definition - The EGIR definition increases sensitivity and specificity in predicting some components of the metabolic syndrome - Prospective collection of breastfeeding data - Many covariates accounted for, such as family history and pubertal stage - Did a sensitivity analysis to analyze whether loss to follow-up influenced the results - Did an observational analysis as well as analyzing the two arms of the trial	- Belarus is an area that has strict hygienic standards, high immunization rates, low incidence of infection, low rates of infant and child morbidity, similar types of infant feeds, and accessible health care services so results may not be generalizable to other populations - Excluded mothers who were unable to breastfeed and preterm or low birth weight babies, which are factors that may predict risk for metabolic syndrome - Much overlap between breastfeeding between both arms, so results may be attenuated - Did not assay HDL-C or triglycerides, which are required for the EGIR criteria used - Used EGIR criteria for diabetics, which apply to non-diabetics only
Sen (2007)	- Detailed physical activity questionnaire - Many important covariates assessed, including family history of diseases and pubertal development - Included formula feeding, which was not included in many of the other studies - Used Oral Glucose Tolerance Test, which can increase accuracy of diagnosing MetS	- Subjects were referred to the Division of Pediatric Endocrinology, so results may not be generalizable to the general populations - Used adult NCEP ATP guidelines for children - Not prospective - Did not measure waist circumference, which is preferred or should be used in conjunction with BMI - Breastfeeding data collected retrospectively
Wang, J (2015)	- High quality control of examinations - Large sample size - Face-to-face interview will improve accuracy of data collection - Used IDF criteria for children and adolescents - Cluster random sampling ensures a more diverse population	- Breast feeding data was obtained retrospectively - Done in a wealthy city, may not be generalizable to poorer populations - All participants were ethnic Han, so can't be generalized to different ethnicities - Not prospective - Used IDF criteria for children under 10. IDF states that children under 10 cannot be diagnosed - Used child and adolescent IDF criteria for those 16 and up
Wang, S (2015)	- The CRF test was a unique and useful covariate in this study - Used IDF criteria in addition to De Ferranti et al. definition, so may catch more cases - Sampling scheme will increase diversity of subjects - Acceptable sample size	- Missing information on physical activity - Using the De Ferranti et al. definition may overestimate prevalence in this population - Not prospective - Missing confounders such as diet, exercise, and family history of other diseases besides HT - Breast feeding history subject to recall bias
Yakubov (2015)	- Biochemical analysis included additional compounds besides the ones needed to diagnosis metabolic syndrome	- Small sample size - Patients' family history was not taken along with other important covariates - Retrospective analysis - Exclusion criteria includes a wide variety of conditions, so results less generalizable

APPENDIX B: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Abstract
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Abstract
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	6-10
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	11
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	12-13
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	42
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	12-13, 42
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	12-13
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	N/A
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	N/A
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	25-28
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	N/A

Appendix B (con't)

Section/topic	#	Checklist item	Reported on page #
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	N/A
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	35
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	N/A
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	12-13, 42
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	N/A
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	23-28
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	28, 46-47
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	N/A
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	N/A
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	N/A
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	30-33
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	34-35
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	35-38
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	N/A

REFERENCES

REFERENCES

1. American Heart Association. About Metabolic Syndrome. http://www.heart.org/HEARTORG/Conditions/More/MetabolicSyndrome/About Metabolic-Syndrome_UCM_301920_Article.jsp. Updated March 9, 2015. Accessed February 12, 2016.

2. National Heart, Lung, and Blood Institute. Department of Health and Human Services. Living With Metabolic Syndome. http://www.nhlbi.nih.gov/health/healthtopics/topics/ms/livingwith. Updated November 6, 2015. Accessed February 12, 2016.

3. Aguilar M, T Bhuket, S Torres, et al. Prevalence of the Metabolic Syndrome in the United States, 2003-2012. *Jama*. 2015;313(19):1973-1974.

4. Cook S, M Weitzman, P Auinger, et al. Prevalence of a Metabolic Syndrome Phenotype in Adolescents. Findings From the Third National Health and Nutrition Examination Survey, 1988-1994. *Arch Pediatr Adolesc Med*. 2003;157(8):821-827.

5. Miller JM, MB Kaylor, M Johannsson, et al. Prevalence of Metabolic Syndrome and Individual Criterion in US Adolescent: 2001-2010 National Health and Nutrition Examination Survey. *Meta Syn and Related Disorders.* 2014;12(10):527-532.

6. Mozundar A and G Liguori. Persistent Increase of Prevalence of Metabolic Syndrome Among U.S. Adults: NHANES III to NHANES 1999-2008. *Diabetes Care*. 2011;34(1):1-4.

7. Petkeviciene J, J Klumbiene, V Kriaucioniene, et al. Anthropometric measurements in childhood and prediction of cardiovascular risk factors in adulthood: Kaunas cardiovascular risk cohort study. *BMC Public Health*. 2015;15:218.

8. Armstrong J, JJ Reilly, and the Child Health Information Team. Breastfeeding andlowering the risk of childhood obesity. *Lancet*. 2003;359:2003-2004.

9. Weyermann M, D Rothenbacher, and H Brenner. Duration of breastfeeding and risk of overweight in childhood: a prospective birth cohort study from Germany. *Int J Obes*. 2006;30:1281-1287.

10. McCrory C and R Layte. Breastfeeding and risk of overweight and obesity at nine-years of age. *Soc Sci Med*. 2012;75:323-330.

11. Jwa SC, T Fujiwara, and N Kondo. Latent Protective Effects of Breastfeeding on Late Childhood Overweight and Obesity: A Nationwide Prospective Study. *Obesity*.2014;22(6):1527-1537.

12. Yan J, L Liu, Y Zhu, et al. The association between breastfeeding and childhood obesity: a meta-analysis. *BMC Public Health*. 2014;14:1267.

13. Yin J, S Quinn, T Dwyer, et al. Maternal diet, breastfeeding and adolescent body composition: a 16-year prospective study. *Eur J Clin Nutr*. 2012;66:1329-1334.

14. Martin RM, D Gunnell, and GD Smith. Breastfeeding in Infancy and Blood Pressure in Later Life: Systematic Review and Meta-Analysis. *Am J Epidemiol*. 2005;161(1):15-26.

15. Owen CG, PH Whincup, K Odoki, et al. Infant Feeding and Blood Cholesterol: A Study in Adolescents and a Systematic Review. *Pediatrics*. 2002;110(3):597-608.

16. Ramirez-Silva I, JA Rivera, B Trejo-Valdivia, et al. Breastfeeding Status at Age 3 Months Is Associated with Adiposity and Cardiometabolic Markers at Age 4 Years in Mexican Children. *J Nutr*. 2015;145:1295-1302.

17. Owen CG, RM Martin, PH Whincup, et al. Does breastfeeding influence risk of type 2 diabetes in later life? A quantitative analysis of published evidence. *Am J Clin Nutr*. 2006;84:1043-1054.

18. Young TK, PJ Martens, SP Taback, et al. Type 2 Diabetes Mellitus in Children. *Arch Pediatr Adolesc Med*. 2002;156:651.

19. Eidelman A and R Schanier. Breastfeeding and the Use of Human Milk. *Pediatrics* 2012;129(3):e827-e841.

20. World Health Organization. *Infant and young child nutrition: Global strategy on infant and young child feeding. Report by the Secretariat.* 2002. Provisional Agenda Item A55/15.

21. Harder T, R Bergmann, G Kallisschnigg, and A Plagemann. Duration of Breastfeeding and Risk of Overweight: A Meta-Analysis. *American Jnl of Epi.* 2005:162(5)397-403.

22. Lawlor DA, CJ Riddoch, AS Page, et al. Infant feeding and components of the metabolic syndrome: findings from the European Youth Heart Study. *Arch Dis Child.* 2005;90:582-588.

23. World Health Organization. Indicators for assessing breast feeding practices. Reprinted report of an Informal Meeting. *WHO/CDD/SER*. 1991;91(14).

24. Grundy SM. Hypertriglyceridemia, Insulin Resistance, and the Metabolic Syndrome. *A J Cardiol*. 1999;83:25F-29F.

25. Grundy SM, HB Brewer, JI Cleeman, et al. Definition of Metabolic Syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association Conference on Scientific Issues Related to Definition. *Circulation*. 2004;109(3):433-438.

26. Miranda PJ, RA DeFronzo, RM Califf, et al. Metabolic syndrome: Definition pathophysiology, and mechanisms. *Am Heart J*. 2005;149:33-45.

27. Eckel RH, SM Grundy, and PZ Zimmet. The metabolic syndrome. *Lancet (London, England)*. 2005;365(9468):1415-1428.

28. Kahn SE, RL Hull, and KM Utzschneider. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. 2006;444(7121):840-846.

29. Grundy SM, JI Cleeman, SR Daniels, et al. Diagnosis and Management of the Metabolic Syndrome: An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation*. 2005;112(17):2735-2752.

30. Mehta NN and MP Reilly. Mechanisms of the metabolic syndrome. *Drug Discov Today Dis Mech*. 2004;1(2):187-194.

31. Park Y, S Zhu, L Palaniappan, et al. The metabolic syndrome: Prevalence and Associated Risk Factor Findings in the US Population From the Third National Health and Nutrition Examination Survey, 1988-1994. *Arch Intern Med*. 2003;163:427-436.

32. Johnson WD, JJM Kroon, FL Greenway, et al. Prevalence of Risk Factors for Metabolic Syndrome in Adolescents. National Health and Nutrition Examination Survey (NHANES), 2001-2006. *Arch Pediatr Adolesc Med*. 2009;163(4):2001-2006.

33. Carnethon MR, CM Loria, JO Hill, et al. Risk Factors for the Metabolic Syndrome: The Coronary Artery Risk Development in Young Adults (CARDIA) study, 1985-2001. *Diabetes Care.* 2004;27(11):2707-2715.

34. Osei K. Metabolic Syndrome in Blacks: Are the Criteria Right? *Curr Diab Rep.* 2010;10:199-208.

35. Freiberg MS, HJ Cabral, TM Heeren, et al. Alcohol Consumption and the Prevalence of the Metabolic Syndrome in the U.S. *Diabetes Care*. 2004;27(12):2954-2959.

36. Wakabayashi I. Frequency of Heavy Alcohol Drinking and Risk of Metabolic Syndrome in Middle-Aged Men. *Alcohol Clin Exp Res*. 2014;38(6):1689-1696.

37. Rajaie S, L Azadbakht, P Saneei, et al. Comparative Effects of Carbohydrate versus Fat Restriction on Serum Levels of Adipocytokines, Markers of Inflammation, and Endothelial Function among Women with the Metabolic Syndrome: A Randomized Cross-Over Clinical Trial. *Ann Nutr Metab*. 2013;63:159-167.

38. Kassi E, P Pervanidou, G Kaltsas, and G Chrousos. Metabolic syndrome: definitions and controversies. *BMC Med*. 2011;9(1):48.

39. International Diabetes Federation. Criteria of Metabolic Syndrome in Children and Adolescents. http://www.idf.org/metabolic-syndrome/children/criteria. Accessed January 11, 2016.

40. Janssen I, PT Katzmarzyk, and R Ross. Waist circumference and not body mass index explains obesity- related health risk. *Am J Clin Nutri*. 2004;79:379-384.

41. Klein S, D Allison, S Heymsfield, et al. Waist Circumference and Cardiometabolic Risk: A Consensus Statement from Shaping America's Health: Association for Weight Management and Obesity Prevention; NAASA, The Obesity Society; the American Society for Nutrition; and the American Diabetes Association. *Obesity*. 2007;15(5):1061-1067.

42. Smith L. New AHA Recommendations for Blood Pressure Measurement. *Am Fam Physician.* 2005;72(7):1391-1398.

43. Grundy SM. Metabolic Syndrome Pandemic. *Arterioscler Thromb Vasc Biol.* April 2008;629-636.

44. Lakka H, DE Laaksonen, TA Lakka, et al. The Metabolic Syndrome and Total and Cardiovascular Disease Mortality in Middle-aged Men. *JAMA*. 2015;288(21):2709-2716.

45. Malik S, ND Wong, SS Franklin, et al. Impact of the Metabolic Syndrome on Mortality From Coronary Heart Disease, Cardiovascular Disease, and All Causes in United States Adults. *Circulation*. 2004;110(10):1245-1250.

46. Reilly JJ and J Kelly. Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: systematic review. *Intern Journ of Obesity*. 2011;35:891-898.

47. Must A. Morbidity and mortality associated with elevated body weight in children and adolescents. *Am J Clin Nutr*. 1996;63(suppl):445S - 447S.

48. Hediger ML, MD Overpeck, RJ Kuczmarski, and WJ Ruan. Association Between Infant Breastfeeding and Overweight in Young Children. *JAMA*. 2001;285(19):2453-2460.

49. Li L, TJ Parsons, and C Power. Breast feeding and obesity in childhood: cross sectional study. *BMJ.* 2003;327(7420):904-905.

50. Owen CG, RM Martin, PH Whincup, et al. The effect of breastfeeding on mean body mass index throughout life: a quantitate review of published and unpublished observational evidence. *Am J Clin Nutr*. 2005;82:1298-1307.

51. Djalalinia S, M Qorbani, R Heshmat, et al. Association of Breast Feeding and Birth Weight with Anthropometric Measures and Blood Pressure in Children andAdolescents: The CASPIAN-IV Study. *Pediatr Neonatol*. 2015;56(5):324-333.

52. Barker DJP. The developmental origins of chronic adult disease. *Acta Paediatr Suppl*. 2004;93(6):26-33.

53. Savino F and SA Liguori. Update on breast milk hormones: Leptin, ghrelin and adiponectin. *Clin Nutr*. 2008;27:42-47.

54. Savino F, MF Fissore, SA Liguori, and R Oggero. Can hormones contained in mothers' milk account for the beneficial effect of breast-feeding on obesity in children? *Clin Endocrinol*. 2009;71:757-765.

55. Cottrell EC and SE Ozanne. Developmental programming of energy balance and the metabolic syndrome. *Proc Nutr Soc*. 2007;66(2):198-206.

56. Hales CN and DJP Barker. The thrifty phenotype hypothesis: Type 2 diabetes. *Br Med Bull*. 2001;60(1):5-20.

57. Palou A and C Picó. Leptin intake during lactation prevents obesity and affects food intake and food preferences in later life. *Appetite*. 2009;52:249-252.

58. Ong KK and DB Dunger. Birth weight, infant growth and insulin resistance. *Eur J Endocrinol*. 2004;151:U131-U139.

59. Ong KK, and RJF Loos. Rapid infancy weight gain and subsequent obesity: systematic reviews and hopeful suggestions. *Acta Paediatr*. 2006;95(8):904-908.

60. Heinig MJ, LA Nommsen, JM Peerson, et al. Energy and protein intakes of breast-fed and formula-fed infants during the first year of life and their association with growth velocity: The DARLING study. *Am J Clin Nutr*. 1993;58(2):152-161.

61. van Deutekom AW, Chinapaw MJM, Vrijkotte TGM, and RJBJ Gemke. The association of birth weight and postnatal growth with energy intake and eating behavior at 5 years of age – a birth cohort study. *Int J Behav Nutr Phys Act*. 2016;13:15.

62. Lifschitz C. Early Life Factors Influencing the Risk of Obesity. *Pediatr Gastroenterol Hepatol Nutr*. 2015;18(4):217-223.

63. St-Onge MP, I Janssen, and SB Heymsfield. Metabolic syndrome in normal-weight Americans: New definition of the metabolically obese, normal-weight individual. *Diabetes Care*. 2004;27(9):2222-2228.

64. Verduci E, G Banderali, S Barberi, et al. Epigenetic effects of human breast milk. *Nutrients*. 2014;6(4):1711-1724.

65. Das UN. A perinatal strategy to prevent coronary heart disease. *Nutrition*. 2003;19(11-12):1022-1027.

66. Cutfield WS, PL Hofman, M Mitchell, and IM Morison. Could epigenetics play a role in the developmental origins of health and disease? *Pediatr Res*. 2007;61(5, Pt 2):68-75.

67. Wang Y and MA Beydoun. The Obesity Epidemic in the United States-Gender, Age, Socioeconomic, Racial/Ethnic, and Geographic Characteristics: A Systematic Review and Meta-Regression Analysis. *Epidemiol Rev.* 2007;29:6-28.
68. Ogden CL, MD Carroll, BK Kit, and KM Flegal. Prevalence of Childhood and Adult Obesity in the United States, 2011-2012. *J Am Med Assoc*. 2014;311:806-814.

69. Heidenreich PA, JG Trogdon, OA Khavjou, et al. Forecasting the Future of Cardiovascular Disease in the United States: A Policy Statement from the American Heart Association. *Circulation*. 2011;123(8):933-944.

70. American Academy of Pediatrics. AAP Policy on Breastfeeding and Use of Human Milk. http://www2.aap.org/breastfeeding/policyonbreastfeedinganduseofhumanmilk.html. Accessed April 18, 2016.

71. Centers for Disease Control and Prevention. Progress in Increasing Breastfeeding and Reducing Racial/Ethnic Differences- United States, 2000-2008 Births. *Morbidity and Mortality Weekly Report.* 2013;62(5):77-80.

72. Centers for Disease Control and Prevention. Breastfeeding Trends and Updated National Health Objective for Exclusive Breastfeeding- United States, Birth Years 2000-2004. *Morbidity and Mortality Weekly Report.* 2007;56(30):760-763.

73. Cai X, T Wardlaw, and DW Brown. Global trends in exclusive breastfeeding. *Int Breastfeed J*. 2012;7(1):12.

74. Ryan AS. The resurgence of breastfeeding in the United States. *Pediatrics*. 1997;99(4):E12.

75. Ford CN, MM Slining MM, and BM Popkin. Trends in Dietary Intake among US 2- to 6-Year-Old Children, 1989-2008. *J Acad Nutr Diet*. 2013;113(1):35-42.

76. Slining MM and BM Popkin. Trends in intakes and sources of solid fats and added sugars among U.S. children and adolescents: 1994-2010. *Pediatr Obes*. 2013;8(4):307-324.

77. Iannotti RJ and J Wang. Trends in physical activity, sedentary behavior, diet, and BMI among US adolescents, 2001-2009. *Pediatrics*. 2013;132(4):606-614.

78. Nelson MC, D Neumark-Stzainer, PH Hannan, et al. Longitudinal and secular trends in physical activity and sedentary behavior during adolescence. *Pediatrics*. 2006;118(6):e1627-e1634.

79. Hamilton BE, JA Martin, MJK Osterman, et al. Births: Final Data for 2014. *National Vital Statistics Report.* Hyattsville, MD: National Center for Health Statistics. 2015;64(1):1-104.

80. Ramadhani MK, DE Grobbee, ML Bots, et al. Lower birth weight predicts metabolic syndrome in young adults: The Atherosclerosis Risk in Young Adults (ARYA)-study. *Atherosclerosis*. 2006;184(1):21-27.

81. Dabelea D, EJ Mayer-Davis, S Saydah, et al. Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. *Jama*. 2014;311(17):1778-1786.

82. Centers for Disease Control and Prevention. Child Development. Atlanta, GA: Division of Human Development and Disabilities, National Center on Birth Defects and Developmental Disabilities. http://www.cdc.gov/ncbddd/childdevelopment/positiveparenting/preschoolers.html. Updated November 5, 2015. Accessed January 13, 2016.

83. World Health Organization. Maternal, newborn, child and adolescent health. http://www.who.int/maternal_child_adolescent/topics/adolescence/dev/en/. Accessed January 13, 2016.

84. Li R, KS Scanlon, and MK Serdula. The Validity and Reliability of Maternal Recall of Breastfeeding Practice. *Nutrition Reviews.* 2005;63(4):103-110.

85. Thompson LA, S Zhang, E Black, et al. The association of maternal pre-pregnancy body mass index with breastfeeding initiation. *Matern Child Health J*. 2012;17(10):1842-1851

86. Mi J, C Law, K-L Zhang, C Osmond, et al. Effects of Infant Birthweight and Maternal Body Mass Index in Pregnancy on Components of the Insulin Resistance Syndrome. *Ann Intern Med.* 2000;132(4):253-260.

87. Boney CM, A Verma, R Tucker, and BR Vohr. Metabolic Syndrome in Childhood: Association With Birth Weight, Maternal Obesity, and Gestational Diabetes Mellitus. *Pediatrics*. 2005;115(3):e290-e296.

88. Jamoussi H, F Mahjoub, H Sallemi, et al. Metabolic syndrome in Tunisian obese children and adolescents. *La tunisie Medicale.* 2012;90(1):36-40.

89. Khuc K, E Blanco, R Burrows, et al. Adolescent Metabolic Syndrome Risk Is Increased with Higher Infancy Weight Gain and Decreased with Longer Breast Feeding. *International Journ of Pediatrics.* 2012;138(6):E6.

90. Wang J, Y Zhu, L Cai, et al. Metabolic syndrome and its associated early-life factors in children and adolescents: a cross-sectional study in Guangzhou, China. [published online ahead of print September 8, 2015]. *Public Health Nutrition.*(doi:10.1017/S1368980015002542).

91. Wang S, Y Liu, J Zhan, et al. Determinants of Metabolic Syndrome in Chinese Schoolchildren. *Asia-Pacific Journ of Public Health.* 2015;27(2):NP674-NP680.

92. Esfarjani F, M Khalafi, F Mohammadi, et al. Metabolic Syndrome and its determination in a sample of young Iranian children with obesity. *Pak J Med Sci.* 2013;29(1):253-257.

93. Sen Y, N Kandemir, A Alikasifoglu, et al. Prevalence and risk factors of metabolic syndrome in obese children and adolescents: the role of the severity of obesity. *Eur J Pediatr.* 2008;167:1183-118

94. Yakubov R, E Nadir, R Stein, and A Klein-Kremer. The Duration of Breastfeeding and Its Association with Metabolic Syndrome among Obese Children. *The Scien World Journ.* 2015;114(3):E4.

95. Ekelund U, S Anderssen, LB Andersen, et al. Prevalence and correlates of the metabolic syndrome in a population-based sample of European youth. *Am J Clin Nutr.*2009;89:90-96.

96. Folic N, M Folic, S Markovic, et al. Risk Factors for the Development of Metabolic Syndrome in Obese Children and Adolescents. *Srp Arc Celok Lek.* 2015;143(3-4):146-152.

97. González-Jiménez E, MA Montero-Alonso, J Schmidt-RioValle, et al. Metabolic syndrome in Spanish adolescents and its association with birth weight, breastfeeding duration, maternal smoking, and maternal obesity: a cross-sectional study. *Eur J Nutr.* 2015;54:589-597.

98. Martin RM, R Patel, MS Kramer, et al. Effects of Promoting Longer Term and Exclusive Breastfeeding on Cardiometabolic Risk Factors at Age 11.5 Years: A Cluster-Randomized, Controlled Trial. *Circulation*. 2014;129(3):321-329.

99. Kramer MS, B Chalmers, ED Hodnett, et al. Promotion of Breastfeeding Intervention Trial (PROBIT): A Randomized Trial in the Republic of Belarus. *JAMA.*2001;285(4):413-420.

100. Bartok CJ and AK Ventura. Mechanisms underlying the association between breastfeeding and obesity. *Intern Journ of Ped Obesity.* 2990;4:196-204.

101. Li R, SB Fein, and LM Grummer-Strawn. Do Infants Fed From Bottle Lack Self-regulation of Milk Intake Compared With Directly Breastfed Infants? *Pediatrics.* 2010;125(6):e1386-e1393.

102. Nommsen-Rivers LA. Does Insulin Explain the Relation between Maternal Obesity and Poor Lactation Outcomes? An Overview of the Literature. Advances in Nutrition: An International Review Journal. 2016 Mar 1;7(2):407-14.

103. De Ferranti SD, K Gauvreau, DS Ludwig, et al. Prevalence of the Metabolic Syndrome in American Adolescents. Findings from the Third National Health and Nutrition Examination Survey. *Circulation.* 2004;110:2494-2497.

104. Bloomgarden ZT. Definitions of the Insulin Resistance. *Diabetes Care*. 2004;27(3):824-830.

105. Weiss R, J Dziura, TS Burgert, et al. Obesity and the Metabolic Syndrome in Children and Adolescents. *N Engl J Med.* 2004;350:2362-2374.