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Critical Review

Adverse Health Outcomes in Offspring Associated With Fetal Alcohol Exposure: A Systematic Review of Clinical and Preclinical Studies With a Focus on Metabolic and Body Composition Outcomes

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Prenatal alcohol exposure (PAE) results in well-characterized neurological, behavioral, and cognitive deficits in offspring. However, the effects on other health outcomes have not been comprehensively described. We used a systematic review methodology to survey published clinical and preclinical studies investigating a broad range of health outcomes in offspring with PAE. This study specifically reports on outcomes related to metabolism and body composition. The literature was systematically searched across 4 electronic databases (PubMed, CINAHL, Embase, and Web of Science), resulting in 3,230 articles following duplicate removal. Titles and abstracts were reviewed against specific inclusion/exclusion criteria, with 242 articles meeting the criteria for full-text assessment of eligibility. Articles with ineligible study type were removed (127) and articles added from reference lists (15) and an updated search closer to submission (9) for a total of 139 studies. Although 5 health domains were identified, here we focus on metabolism and body composition. Details of alcohol exposure, offspring demographics, and sample sizes were tabulated and quality of reporting assessed. Findings were summarized for body composition (percentage fat mass), physiological and molecular outcomes related to glucose metabolism, and outcomes related to lipid metabolism. There were 32 included studies (2 case–control, 1 prospective longitudinal cohort, and 29 preclinical). Studies had a range of alcohol exposures, both dosage and timing, although all clinical studies had heavy PAE and/or evidence of fetal alcohol syndrome in offspring. The preclinical studies provided evidence of glucose intolerance and/or insulin resistance; dyslipidemia and/or hypercholesterolemia; and increased adiposity in offspring with PAE. Due to the paucity of clinical studies, we recommend further studies be conducted to obtain a complete assessment of long-term metabolic health outcomes in children and adults with PAE, particularly in those diagnosed with fetal alcohol spectrum disorder.

Key Words: FASD, Fetal Alcohol Exposure, Glucose Tolerance, Insulin Sensitivity, Metabolic Syndrome.

THERE IS NOW abundant evidence, from both clinical cohorts and animal studies, suggesting that the maternal environment is critical in setting the trajectory for future offspring health. This is known as the developmental origins of health and disease (DOHaD) hypothesis (Barker, 2007) and implicates prenatal perturbations such as maternal malnutrition, stress, obesity, and drug use toward an increased risk for chronic diseases in offspring, such as diabetes and coronary heart disease, in later life. One such potential perturbation that has not received as much attention is prenatal alcohol exposure (PAE). Recent data from the United States estimate that in the 3 months preceding pregnancy, 54% of women consumed alcohol while 25% were obese and 19% smoked (Robbins et al., 2014). Given that 50% of pregnancies are unplanned, this highlights that alcohol exposure, particularly around conception and early pregnancy, is likely to be more common than many other well-recognized maternal risk factors for pregnancy complications and poor fetal outcomes, such as smoking or obesity.

Research into the adverse effects of PAE has typically focused on neurological and behavioral outcomes in offspring. These outcomes form the basis of fetal alcohol spectrum disorder (FASD), the term that has been adopted to include the broad spectrum of disabilities that can result for children with PAE, including physical, cognitive, behavioral, emotional, and social difficulties (Lange et al., 2017).

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However, there is increasing interest in the effect of PAE on other organs and systems and the potential increased risk for developing chronic conditions such as cardiovascular disease and type 2 diabetes. This was highlighted by a panel of young adults with FASD at the Seventh International FASD Conference (2017), who stated that rather than describing FASD as a brain-based disorder, it should be described as a "whole-body disorder" (Himmelreich et al., 2017). This conference also prompted publication of a minireview, highlighting that even low–moderate alcohol exposure can program chronic disease in offspring, at least from animal studies (Sarman, 2018).

Reviews summarizing the effects of PAE, not specifically related to cognitive and behavioral outcomes, nevertheless are often brain-based. For example, alterations to the hypothalamus and/or pituitary gland have been implicated in changes to immune function (Taylor et al., 2006; Zhang et al., 2005), circadian rhythms and sleep patterns (Inkelis and Thomas, 2018), and neuroendocrine function (Akison et al., 2019) in offspring with PAE. PAE has also been reported to affect the neurological control of breathing due to reduced nerve output from the brain stem (Dubois et al., 2013). However, there have been relatively few reviews of the impacts of PAE on other organs and body systems. Caputo and colleagues (2016) included 5 clinical studies that reported on abnormalities in the heart, kidney, liver, gastrointestinal tract, and endocrine system, but they also included brain-based outcomes. A descriptive review by Lunde and colleagues (2016) discussed the potential of prenatal alcohol to contribute to developmental programming of chronic diseases in adulthood and included evidence from preclinical studies. In terms of studies looking specifically at metabolic outcomes, Ting and Lautt (2006) provide a descriptive review of the effects of acute, chronic, and PAE on insulin sensitivity, while Vaiserman (2015) reported on early-life exposure to various forms of substance abuse, including alcohol, and the evidence for increased risk to develop type 2 diabetes in adulthood.

No previously published reviews have used a systematic review methodology, which is an explicit, prespecified, and reproducible method of identifying, appraising, and synthesizing the evidence allowing a reliable summary to guide future research. Previous reviews have also not incorporated both clinical and preclinical studies. Animal studies have played an important role in confirming the teratogenic effects of alcohol and elucidating the mechanisms of these effects, as researchers can control a range of factors that could influence outcomes, including the dose and timing of the exposure. Clinical studies are important to consider the real-world application of preclinical results and thus, in combination, will assist to integrate and highlight gaps in the current research. Therefore, this review aimed to conduct a systematic analysis of the available clinical and preclinical studies that describe nonneurological health outcomes in offspring following PAE. Given that malformations and growth deficits are well-known features of PAE (Del Campo and Jones, 2017; Viteri et al., 2015), studies exclusively reporting on these were excluded. While we briefly report the overall findings in the current review regarding the range of health outcomes identified, this paper specifically summarizes the literature on the metabolic and body composition impacts of PAE. We chose this as the focus for 2 reasons: Firstly, the evidence from the DOHaD field is very strong for the programming of diabetes and obesity following a suboptimal in utero environment, and secondly, although growth deficiency is a defining feature of fetal alcohol syndrome (FAS), there is growing concern that individuals with other diagnoses on the FASD spectrum may be at increased risk of overweight or obesity compared to the population. All other studies were grouped by domain and included as a summary of the breadth of adverse health impacts in offspring exposed to alcohol during development.

MATERIALS AND METHODS

Search Strategy

Articles were identified through a systematic search of the following computerized bibliographic databases: PubMed, CINAHL, Web of Science, and Embase from inception to December 2017. An updated search was conducted in October 2018, and any additional studies identified that met the search criteria were included. Search terms were as follows: maternal OR prenatal OR neonatal OR fetal OR foetal OR pregnancy OR pregnant OR fetal programming AND alcohol OR ethanol (EtOH) OR fetal alcohol OR foetal alcohol AND renal OR kidney OR cardiac OR heart OR cardio OR metabolic OR diabetes OR obesity OR respiratory OR lung OR immune OR reproductive OR endocrine. Search terms were uniformly applied across all 4 databases. The systematic review was registered with the international prospective register of systematic reviews (PROSPERO, CRD42017082627), accessible online at http://www.crd.york.ac.uk/PROSPERO/.

The initial search and removal of duplicates was performed by author NR. Screening of titles and abstracts was performed by authors NR and LA. Abstracts meeting inclusion criteria or those requiring the full text to clarify inclusion were retained and reviewed independently by 2 authors (NR, LA). Consensus was reached by discussion between the authors, and articles were referred to a third author (KM) where any disagreements about eligibility arose. Reference lists of on-topic reviews retrieved from the initial search were manually searched to identify additional relevant publications.

Study Selection Criteria

Studies were included if they met the following criteria: (i) animal/in vivo study (any mammal) with fetal alcohol exposure; (ii) alcohol exposure occurred during pregnancy or during the third trimester–equivalent period in the rat (up to postnatal day 9); (iii) for preclinical studies, alcohol concentration/dose and accurate timing of exposure must be provided (any route of administration); (iv) for clinical studies, children diagnosed with FASD using specified diagnostic criteria or suspected to have been exposed to moderate-to-heavy PAE via a stated method of maternal alcohol assessment; (v) a physical health impact of PAE was assessed in offspring (e.g., renal, cardiac, metabolic, reproductive, immune); and (vi) included an appropriate control group for comparison. Studies were excluded if they: (i) were not published in English; (ii) only included assessments related to brain-based, neurological, or behavioral outcomes; (iii) only included outcomes related to malformations, growth rates, or growth restriction; (iv) only documented effects on

embryos, fetuses, or neonates during the third trimester–equivalent period in the rat; (v) only focused on maternal effects of alcohol exposure rather than offspring effects; and (vi) were conference abstracts, PhD dissertations, or other editorials.

Study Quality Assessment

The methodological quality of included studies was scored by 2 independent assessors (MW, LA). For clinical studies, an adapted version of the Downs and Black Checklist (Downs and Black, 1998) was used, which is an instrument that evaluates study quality in the following categories: (i) reporting, (ii) external validity, (iii) internal validity, and (iv) statistical power. We adapted the checklist to remove items related to intervention studies, resulting in 20 items that provided a maximum possible score of 19. For preclinical studies involving animal models, the animal research: reporting of in vivo experiments (ARRIVE) guidelines were used to assess study quality (see Kilkenny and colleagues 2010 for full details of criteria). The ARRIVE guidelines consist of a checklist of 20 items, resulting in a maximum score of 40. The checklist allows assessment of the quality of reporting of information such as the species/strain, source, and husbandry of animals; welfare and adverse events; treatments and experimental design; anesthesia/analgesia and method of euthanasia; and analysis. After discussion between assessors, consensus was reached on the final quality scores.

Data Extraction and Synthesis

Information about the study design, sample characteristics, and outcomes measured were extracted and summarized for each of the included studies by authors MW and LA. This included details of the study type (clinical/preclinical), species, alcohol exposure/treatment, and offspring sample sizes/characteristics at assessment. Sample sizes were estimated when accurate numbers per group were not available. Information for preclinical studies was organized based on the timing of the alcohol exposure (i.e., stage of pregnancy). Nonexposed offspring were considered controls. Descriptive information was obtained from each study on the relevant assessments used, postnatal insults or "second hits" (e.g., high-fat diet [HFD], stress), key results, and major conclusions. Where data were analyzed separately by sex, any sex-specific differences were noted.

RESULTS

Search Results and Classification of Included Studies by Health Outcomes

Database searching identified 3,230 abstracts after duplicates were removed (Fig. 1). These were then screened against inclusion/exclusion criteria, resulting in retrieval of 242 full publications for more detailed investigation. Reasons for exclusion of studies are detailed in the study flow diagram (Fig. 1). Of the 242 full-text publications retrieved, 93 were conference abstracts, 24 were on-topic reviews, and 10 were not the correct article type (i.e., editorial, conference proceedings) and so were excluded (Fig. 1). On-topic reviews and included paper reference lists provided an additional 15 publications, while an updated search closer to submission identified an additional 9 publications (Fig. 1). Therefore, a total of 139 articles were included in the review.

All but 1 article could be classified under 5 health domains as shown in Fig. 1. Publications relating to 4 of these domains (cardiovascular/renal, reproductive, liver/intestinal, and immune function) will form the basis of other publications but are included for reference in Data S1. The 1 study that could not be classified was by Probyn and colleagues (2013a), which reported on the effects of PAE on the structure and function of the lung in a rat model (details also included in Data S1). Finally, there were 32 studies reporting on outcomes related to metabolism (glucose and/or lipid) and body composition. Of these, 3 were clinical studies, while preclinical studies were in rodent models. These papers form the basis of this review, and details are reported below.

Quality of Methodological Reporting in Studies Examining Offspring Metabolism and Body Composition

Assessment of the 3 clinical studies using the adapted Downs and Black (1998) criteria revealed a distinct mismatch in the quality of reporting, with the older study by Castells and colleagues (1981) resulting in a total score of 6 out of a possible 19 (Data S2A). This study failed to report patient information for the control group or demonstrate attempts to control external and internal validity and conducted no formal statistics to compare PAE to control outcomes. Therefore, the quality of reporting for this study was very low, but given the paucity of clinical studies in this area, it was still included in this review. In contrast, Carter and colleagues (2012) and Amos-Kroohs and colleagues (2016) had much higher total scores of 16 and 15, respectively, with consistent loss of points for: (a) failing to provide information on how representative the study participants were of the entire population of patients from which they were recruited (items 11 and 12); and (b) not conducting a post hoc analysis of the statistical power of the study based on the actual number of participants recruited (Data S2A).

For preclinical studies, ARRIVE scores ranged from 16 to 32 out of a possible 40 (Data S2B), indicating a wide range of quality of reporting across studies (Fig. 2). Score deductions mostly related to missing methods for overcoming bias, lacking explanations for why particular methods or treatment regimens were chosen, lack of baseline information on the dams prior to treatment, no information on how sample sizes were determined (e.g., power analysis), inaccurate reporting of sample sizes, no methods stated to test whether data met the assumptions of statistical tests, no justification for exclusion of data from analyses, and no information on adverse events encountered during experiments. With regard to sample sizes, often the number of dams treated was clearly stated, but it was unclear how many offspring from each dam were included in each analysis.

Description of Studies Examining Offspring Metabolism and Body Composition

Details of the studies reporting on metabolic outcomes and body composition are summarized in Table 1. The clinical study by Castells and colleagues (1981) was a small case–control study that included 7 children diagnosed with FAS

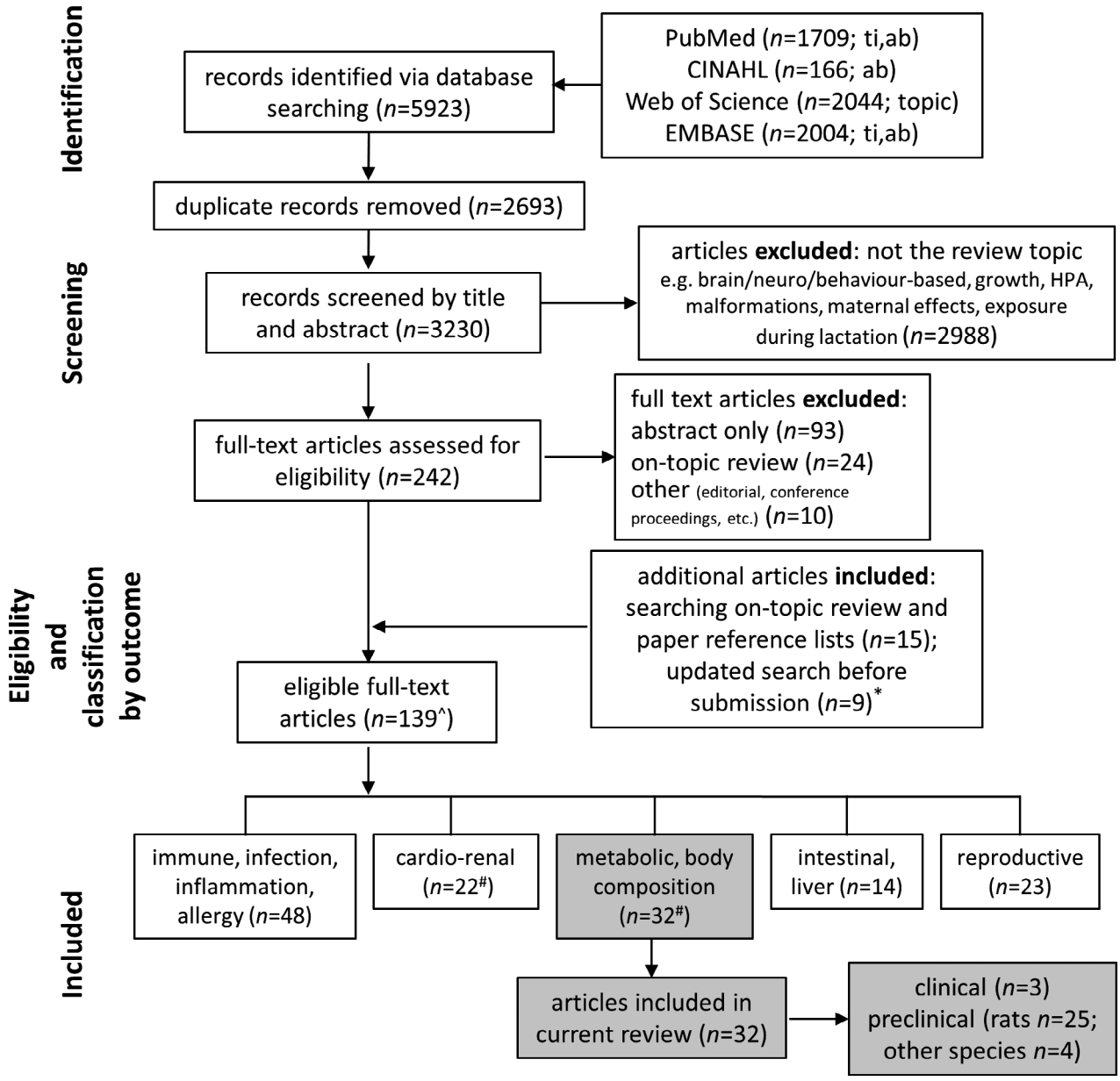

Fig. 1. Flow diagram of the literature search strategy and study selection process (based on the PRISMA statement [Moher et al., 2009]). Studies were grouped by specific health outcomes examined in offspring exposed to alcohol during development. Data extraction and reporting was specifically done only on studies reporting on metabolic health outcomes and body composition/obesity in the current review. Studies from other domains are listed in Data S1. *Search repeated in October 2018. ^Only one study reported on structural and functional deficits in the lung and so is not classified to a group and is only discussed in the text. #One of the papers contains data on both metabolic and cardiorenal outcomes and so is counted in both of these groups. ti = title; ab = abstract.

and 20 control children. No information was provided for the control participants other than aggregate results following a glucose tolerance test (GTT). Carter and colleagues (2012) was a more recent prospective longitudinal cohort based in a heavy-drinking, economically disadvantaged, Cape Colored community in Cape Town, South Africa. Women were recruited from an antenatal clinic of a midwife obstetric unit. Amos-Kroohs and colleagues (2016) was a case–control study, with participants diagnosed with FASD recruited through the University of Minnesota's FASD program and compared against typically developing controls. All clinical studies reported growth restriction at birth and reported primarily on early–school-age children (6 to 9 years).

However, the majority of studies in this domain were preclinical studies, primarily in the rat (Table 1). A range of offspring ages was assessed, from preweaning (PD10–PD20), immediately postweaning (PD30), to adulthood (12 weeks to 12 months of age). Some studies examined only 1 sex (7 studies for male and 3 studies for female), while the majority included both sexes, often splitting the analysis to test for sex differences. Sample sizes per group were variable but

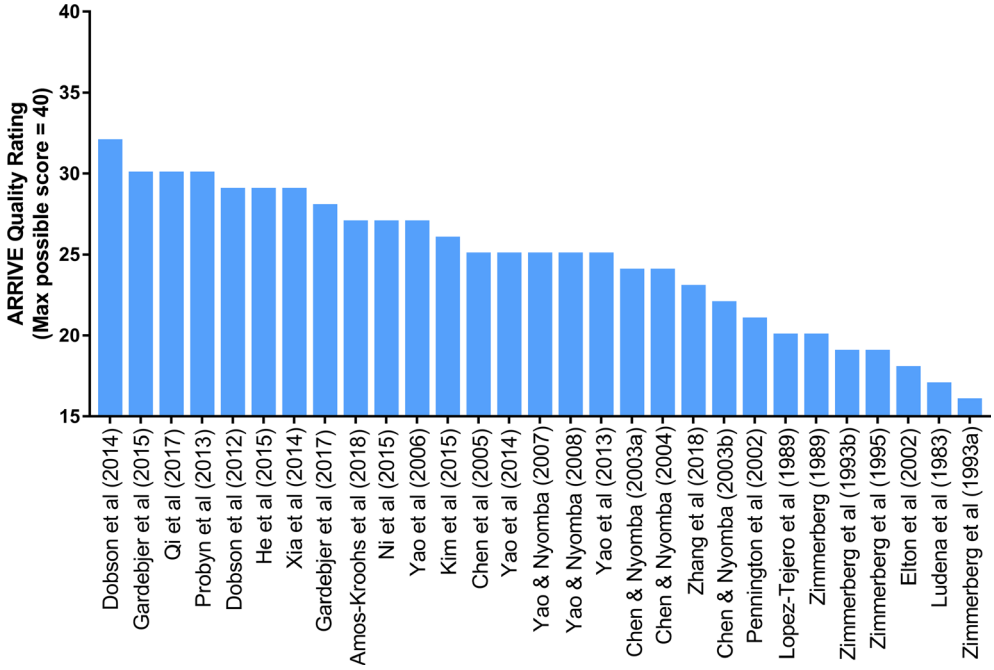

Fig. 2. Total quality assessment scores for the preclinical studies based on the ARRIVE guidelines (Kilkenny et al., 2010), ordered from highest quality of reporting to lowest. See Data S2B for details of how scores were tallied. Highest possible score is 40.

predominantly low ($n < 10$), and in many cases had to be estimated due to poor reporting of accurate sample sizes (see also Data S2B). Although sometimes approximate, we believe the sample size estimation provides a crude measure of potential statistical power that can be used to compare among studies. Several studies appeared to report on offspring from the same treated dams used in other studies (see Table 1 for details), so, although there were 29 studies included, we estimate that they collectively refer to 18 independent sets of treated dams. Some studies also included a postnatal challenge or "second Hit" This was often exposure to a HFD, which ranged from 19 to 60% calories from fat (regular chow is typically ≤10% calories from fat). Where a "second Hit" exposure was included in the study, details of this were provided in the appropriate outcome table.

Details of Alcohol Exposure

Alcohol exposure was determined at recruitment for clinical studies by self-reported heavy prenatal alcohol use and/or FAS/FASD diagnosis (see Table 1 for details). Detailed reporting on maternal drinking was lacking for Castells and colleagues (1981), but all 7 children included in the study had facial dysmorphology, microcephaly, low birthweight, and poor coordination and were diagnosed with FAS based on a description of principal clinical features available at that time (Clarren and Smith, 1978). Therefore, it is assumed that there was heavy exposure in the group with PAE. For the Carter and colleagues (2012) study, women were asked about their alcohol consumption at the time of conception and recruitment using an interview derived from the timeline follow-back approach (developed by Jacobson et al., 2002). Women averaging 2 standard drinks per day (equivalent to 1 oz absolute alcohol) or at least 2 binge-drinking episodes (≥5 standard drinks per occasion) during the first trimester of pregnancy were recruited into the study. Women in the PAE group averaged 2.1 drinking days per week around conception and 1.5 days per week during pregnancy, with an average of ~4 oz absolute alcohol (8 standard drinks) per drinking day at both time points. Therefore, although the number of drinking days was reduced after recognition of pregnancy, this reflects binge-drinking behavior throughout pregnancy. FAS/pFAS diagnosis was undertaken using the revised Institute of Medicine criteria (Hoyme et al., 2005), with 21% of children with PAE diagnosed with FAS and 27.2% diagnosed with pFAS. Amos-Kroohs and colleagues (2016) used the same criteria for diagnosis, but also applied the Centers for Disease Control and Prevention central nervous system criteria for FASD (Centers for Disease Control and Prevention, 2005). In their study, 28.4% were diagnosed with FAS and 28.4% with pFAS. For preclinical studies, the timing of alcohol exposure varied across the prenatal period, with some studies treating dams throughout pregnancy, while others treated for distinct windows either early, mid, or late in gestation (Table 1; Fig. 3). Only 2 studies by Gårdebjer and colleagues (2015, 2017) treated specifically around the periconceptional period, which included exposure for 1 estrous cycle prior to mating/conception and for the first 4 days of pregnancy, prior to implantation. Ludena and colleagues (1983) and Lopez-Tejero and colleagues (1989) also included a preconception exposure period of 4 weeks.

Table 1. Participant and Study Characteristics

Study	Study type: species	Alcohol exposure	Isocaloric control	Offspring assessed				
				Age	Sex	Control (*n*)^	EtOH (*n*)^	Growth restricted+
Clinical								
Amos-Kroohs and colleagues (2016)	Case–control study	FASD diagnosis[mm]	–	8 to 9 years[oo]	F/M	48	72	Yes[nn]
Carter and colleagues (2012)	Prospective longitudinal cohort	First trimester reported heavy PAE[a]	–	9 years	F/M	54	74[b]	Yes
Castells and colleagues (1981)	Case–control study	FAS diagnosis[c]	–	6 to 7 years[d]	FM	20[e]	7	Yes
Preclinical								
Periconceptional[f] and early gestation (to GD8)								
Gårdebjer and colleagues (2015) (GD-4 toGD4)[g]	Preclinical: rat	12.5% v/v (~25% EDC) in liquid diet[h]	Yes	6 to 8 months	F/M	7 to 12	7 to 12	Yes[i]
Gårdebjer and colleagues (2017) (GD-4 toGD4)[g]								
Yao and colleagues (2013)[j] (GD1–GD7)	Preclinical: rat	4 g/kg/d via gavage[k]	Yes	16 weeks	M	6	6	No[l]
Zhang and colleagues (2018) (GD0–GD8)	Preclinical: mouse	10% v/v in drinking water	No	12 and 21 to 26 weeks	F/M	10 to 12	9 to 16	NR
Preconception (4 weeks) and throughout gestation								
Lopez-Tejero and colleagues (1989)	Preclinical: rat	25% w/v in drinking water[m]	No	PD15, PD30, PD90	F/M	5 to 10	5 to 10	Yes
Ludena and colleagues (1983)	Preclinical: rat	30% w/v in drinking water[n]	No	PD15	NR	8 to 10	8 to 10	Yes
Throughout gestation								
Chen and Nyomba (2003a)[o]	Preclinical: rat	4 g/kg/d via gavage[k]	No	13 weeks	M[p]	3 to 6	3 to 6	Yes
Chen and Nyomba (2003b)[o]								
Chen and Nyomba (2004)[q]			No	16 weeks	M	5	5 to 8	No[r]
Chen and colleagues (2005)[q]						4	4	
Yao and colleagues (2006)			No	12 to 14 weeks	M	6 to 12	6 to 12	Yes
Yao and Nyomba (2007)[s]			Yes	12 to 13 weeks	F	6	6	No
Yao and Nyomba (2008)[s]								
Dobson and colleagues (2012)[v]	Preclinical: guinea pig	8 g/kg/d (30% v/v) per os[t]	Yes	PD100–PD200	F/M[w]	10 to 16[u]	8 to 12	Yes
Dobson and colleagues (2014)[v]						4 to 24[u]	5 to 15[u]	
Elton and colleagues (2002)[x]	Preclinical: rat	35% EDC in liquid diet[h,y]	Yes	PD60–PD360	F/M	3 to 6	3 to 6	Yes
Pennington and colleagues (2002)[x]						6	6	
Probyn and colleagues (2013b)	Preclinical: rat	6% v/v (15% EDC) in liquid diet[h]	Yes	PD30; 2 to 8 mths	F/M	7 to 15	7 to 15	No[z]
Early gestation (GD6/7) to birth								
Kim and colleagues (2015)[aa]	Preclinical: rat	6.7% v/v liquid diet (35% EDC)[h,bb,cc]	Yes	PD60–PD70	F/M	9 to 13	9 to 13	NR
Zimmerberg (1989)[aa]	Preclinical: rat	35% EDC in liquid diet[h]	Yes	PD10–PD20	FM	6	6	Yes
Zimmerberg and colleagues (1993a)[aa,dd]				PD20	F/M	3[ee]	3[ee]	
Zimmerberg and colleagues (1993b)[aa,dd]				PD10, PD20	FM[ff]	12 to 15	12 to 15	
Zimmerberg and colleagues (1995)[aa]				PD10, PD20	FM[ff]	12[gg]	12[gg]	
Mid- to late gestation								
Amos-Kroohs and colleagues (2018) (GD12.5–GD17.5)	Preclinical: mouse	3 g/kg/d via gavage[hh]	Yes	17 to 23 weeks	F/M	8 to 12	8 to 12	No
Yao and colleagues (2013)[j] (GD8–GD14)	Preclinical: rat	4 g/kg/d via gavage[k]	Yes	16 weeks	M	6	6	Yes
Mid-gestation (GD11) to birth								
He and colleagues (2015)	Preclinical: rat	4 g/kg/d via gavage[k]	No	20 weeks	F/M	8	8	Yes[jj]
Ni and colleagues (2015)				24 weeks	F	5	5	
Qi and colleagues (2017)				24 weeks	F/M	8	8	
Xia and colleagues (2014)				20 weeks	M	8	8	
Late gestation (GD15) to birth								
Yao and colleagues (2013)[j]	Preclinical: rat	4 g/kg/d via gavage[k]	Yes	16 weeks	M	6	6	Yes
Yao and colleagues (2014)[kk]			Yes	16 weeks	F	6 to 14[ll]	6 to 14[ll]	Yes

The clinical studies are shown first ($n = 3$), followed by the preclinical studies ($n = 29$). Animal studies are ordered based on the timing of alcohol exposure (summarized in Fig. 3).

EDC, EtOH-derived calories; EtOH, ethanol; F, females only; F/M, females and males separated in analyses; FAS, fetal alcohol syndrome; FASD, fetal alcohol spectrum disorder; FM, females and males combined in all analyses; GD, gestational day; M, males only; mths, months; NR, not reported; PD, postnatal day; v/v, volume/volume.

^Sample sizes are per outcome measured. Where analyses were split by sex, *n* reflects sample size per sex per treatment/control.

+At birth.

[a]> 1.0 oz AA/day or ≥ 2.5 oz AA/occasion (≥2 occasions); AA = absolute alcohol (1.0 oz AA equivalent to 2 standard drinks).

[b]Only *n* = 37 had a confirmed diagnosis of FAS or partial FAS (pFAS). *n* = 37 were confirmed as not FAS or pFAS.

[c]FAS diagnosis based on maternal history of alcohol consumption during pregnancy, characteristic facial dysmorphology, and neurological impairment as described by Clarren and Smith (1978).

[d]One child was 18 months old.

[e]No information provided on age or sex of controls.

[f]One estrous cycle prior to mating to GD4.

[g]Offspring from the same treated dams.

[h]Lieber–DeCarli-style diet (DeCarli and Lieber, 1967).

[i]Previously reported by Gårdebjer and colleagues (2014).

[j]Yao and colleagues (2013) treated subsets of animals at GD1–GD7 (early), GD8–GD14 (mid), and GD15–birth (late).

[k]2 g/kg (36% v/v) EtOH via twice-daily gavage at 0900 and 1600 (7 hours apart).

[l]Growth restriction only reported for groups exposed to EtOH in mid–late gestation.

[m]Alcohol given for 4 weeks prior to mating and throughout gestation; initial dose 10% w/v EtOH for the first week, then weekly increase by 5% to final dose of 25% w/v EtOH greater than or equal to fourth week.

[n]Alcohol given for 4 weeks prior to mating and throughout gestation; initial dose 10% w/v EtOH for the first week, 5% increase in week 2, 10% increase in week 3 and final dose of 30% w/v EtOH greater than or equal to fourth week.

[o]Offspring from the same treated dams.

[p]Epididymal fat was collected with other tissues for molecular analysis so assumed to be males.

[q]Only outcomes for prenatal alcohol exposure are reported. Offspring are from the same treated dams.

[r]A subset of EtOH-exposed pups were reported as small for gestational age (although not statistically different to unexposed pups) while the rest were normal-weight. For Chen and Nyomba (2004), analyses were split into 2 different birthweight groups. Not stated which pups were used for the Chen and colleagues (2005) paper.

[s]Offspring from the same treated dams.

[t]4 g/kg via twice-daily dosing (2 hours apart) into the oral cavity via syringe, 5 days per week.

[u]Dobson and colleagues (2012) reports that *n* = 12 dams were treated in each group. Therefore, where sample sizes are reported as >12 for a particular outcome, more than 1 animal of each sex was used per litter. The authors comment in methods that previous studies show that within-litter variability is similar to between-litter variability in this model.

[v]Offspring from the same treated dams.

[w]If no effect of sex on a particular outcome, data were pooled across sex for analysis.

[x]Suspect offspring from the same treated dams (*n* = 72 to 75 total, including controls). Offspring sample size reporting for both studies was unclear and inconsistent and is therefore approximate.

[y]Alcohol content increased over 4 days to final concentration in Pennington et al.; starting concentration not specified. Note that the EtOH concentration was only mentioned in the abstract for Elton and colleagues as "35% of calories as EtOH" but was not explicitly stated in the methods or anywhere else in the paper. Alcohol was added to a liquid diet of 12% or 35% calories from fat; therefore, some dams had the added insult of HFD (Elton and colleagues only).

[z]Previously reported by Probyn and colleagues (2012).

[aa]Outcomes for these studies have been mentioned in the text only.

[bb]EtOH concentration increased from 2.2% v/v on day 7 to 4.4% v/v on day 8 to a final concentration of 6.7% by day 9.

[cc]Liquid diet contained 36% calories from fat, which is considered a HFD maternal insult by Elton and colleagues (2002).

[dd]Offspring from the same treated dams.

[ee]Each sample made up of pooled tissue from 3 same-sex, same-age animals.

[ff]Analysis was presented pooled across sex as there was no significant effect of sex (data not shown).

[gg]Each sample made up of pooled tissue samples from an unknown number of pups. *n* = 6 per sex for each group, with analysis pooled across sex.

[hh]1.5 g/kg via twice-daily gavage (2 hours apart).

[ii]Suspect offspring from the same treated dams.

[jj]Reported in Shen and colleagues (2014).

[kk]Suspect offspring from the same treated dams as those used in Yao and colleagues (2013).

[ll]Sample sizes not precisely given.

[mm]Diagnosed using modified Institute of Medicine criteria (Hoyme et al., 2005) and Centers for Disease Control and Prevention (2005) central nervous system criteria for FASD. EtOH group: *n* = 21 with FAS; *n* = 21 with pFAS; *n* = 26 with alcohol-related neurodevelopmental disorder (ARND); *n* = 6 with possible FASD.

[nn]A significantly greater percentage of children with PAE were reported as small for gestational age at birth (6.2 vs. 2.5%).

[oo]Average age given; range 2.5 to 17 years.

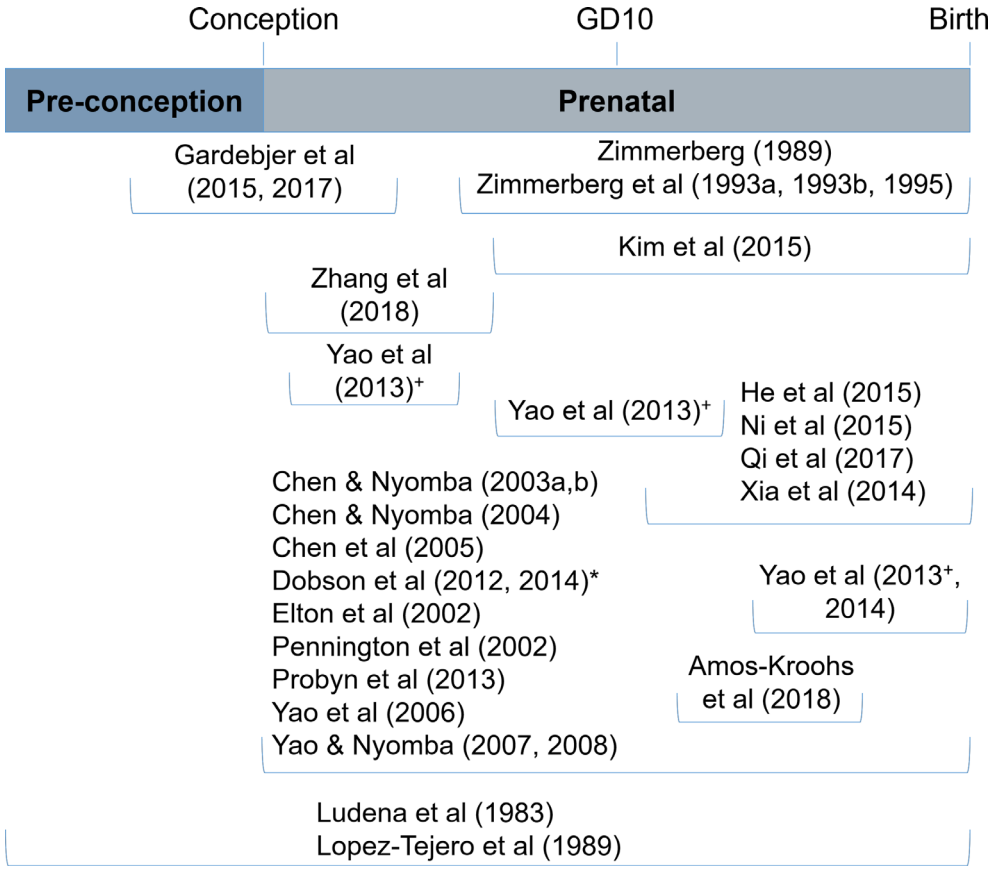

Fig. 3. Summary of included preclinical studies relative to the timing of alcohol exposure. Further details are provided in Table 1. + Yao and colleagues (2013) had 3 subsets of EtOH-treated dams at early, mid-, and late gestation. * Dobson and colleagues (2012, 2014) were guinea pig studies, so gestation is 60 days.

Alcohol was administered either via gavage, in drinking water, or in a Lieber–DeCarli-style liquid diet (i.e., nutritionally complete liquid diet, no chow provided; Table 1). Alcohol dosages were relatively consistent across studies. For studies administering EtOH via gavage (or *per os*), dosages ranged from 3 g/kg/d (mouse) to 4 g/kg/d (rat) to 8 g/kg/d (guinea pig). All studies split these into 2 equal doses given twice daily; therefore, maximum dose per gavage was 1.5, 2, or 4 g/kg, respectively. Blood alcohol concentration (BAC) 2 hours after gavage with 2 g/kg EtOH was reported in the range 100 to 150 mg/dl (Chen and Nyomba, 2003a; 0.1 to 0.15%), which equates to ~3 to 5 standard drinks (~1.5 to 2.5 oz) consumed by a woman in 2 hours (Leeman et al., 2010). By 4 hours postgavage, BAC had reduced to 70 mg/dl (Chen and Nyomba, 2003a; 0.07%). The guinea pig studies resulted in a BAC of 281 ± 15 mg/dl (0.28%) ~1 hour after the second daily dose (Dobson et al., 2012). For studies administering EtOH via an ad libitum liquid diet, concentrations ranged from 6% v/v to 12.5% v/v (see Table 1). For the low-dose (6% v/v) model reported by Probyn and colleagues (2013b), an earlier study quantified average consumption rates of diet and estimated that the EtOH dosage was ~0.21 g/kg/h (Probyn et al., 2012). This resulted in a peak BAC of 0.03% 0.5 hour after fresh diet was first offered for the day, but by 1 hour, BAC was negligible (Probyn et al., 2012). The 12.5% v/v diet reported by Gårdebjer and colleagues (2015, 2017) resulted in a peak BAC of ~0.18 to 0.25% 0.5 hour after fresh diet was offered, dropping to ~0.07% at 3 hours and ~0.05% at 5 hours as reported previously (Gårdebjer et al., 2014). Some studies reported the concentration as percentage of EtOH-derived calories (EDC), with most studies providing 35% EDC. This resulted in a similar peak BAC to the gavage studies (100 to 150 mg/dl; Elton et al., 2002). Only 2 studies administered EtOH via the drinking water (25 to 30% w/v; Lopez-Tejero et al., 1989; Ludena et al., 1983), with this equating to ~30 to 35% EDC (Lopez-Tejero et al., 1989). No BACs were reported in any of the studies where EtOH was delivered in water. No studies reported birth defects, abnormal number of pups, skewed sex ratios, or miscarriages at these doses.

Details of Control Groups

We aimed to include only studies with a no–alcohol exposure control group in this review. For the clinical studies, Castells and colleagues (1981) did not provide any information on their control group, aside from the sample size and stating that they were "normal" controls. Amos-Kroohs and colleagues (2016) reported that none of their typically developing control participants had PAE, with 65% recruited

through the University of Minnesota's FASD program and the rest being siblings of children recruited for an unrelated study at the University of Wisconsin-Madison. Carter and colleagues (2012) recruited their control group from the same source population, with women reporting drinking <0.5 oz absolute alcohol/day (1 standard drink) and no binge drinking during the first trimester being invited to participate as abstainers/light drinkers. However, 6 women initially recruited as light drinkers reported heavy drinking in prospective interviews during pregnancy and were reclassified as heavy drinkers. Two women who initially denied drinking had children diagnosed with FAS and either acknowledged heavy drinking retrospectively or had high meconium levels of fatty acid ethyl esters, indicating heavy exposure (Bearer et al., 2003). Women in the final control group averaged 0.1 drinking days per week around conception and during pregnancy, and 0.1 to 0.5 oz absolute alcohol (0.2 to 1 standard drinks) per drinking day. Given the lack of clinical studies in this domain, we accepted the negligible rather than zero consumption rate for the controls in this study.

All included preclinical studies had a no–alcohol exposure control group. However, there were a variety of ways that researchers provided an isocaloric alternative for the control group in lieu of EtOH treatment (Table 1). Probyn and colleagues (2013b) and Gårdebjer and colleagues (2015, 2017) adjusted their control liquid diet formulas to account for extra calories provided by EtOH or different consumption rates, respectively. Zimmerberg and colleagues (Zimmerberg, 1989; Zimmerberg et al., 1993a,1993b, 1995) used a pair-feeding approach, by including control dams individually "yoked" to an alcohol group dam, which received the same volume of liquid diet with maltodextrin substituted for EtOH. Elton and colleagues (2002), Pennington and colleagues (2002), and Kim and colleagues (2015) used a similar isocaloric pair-feeding strategy without maltodextrin substitution. Amos-Kroohs and colleagues (2018) included control dams gavaged with an isocaloric maltodextrin or medium-chain triglyceride solution, as well as an equivalent-volume water control. Dobson and colleagues (2012, 2014) included a control group where each individual was paired to an EtOH-treated animal and received isocaloric sucrose (*per os*) and chow in the amount consumed daily by the EtOH-treated animal. Yao and colleagues (2013, 2014) and Yao and Nyomba (2007, 2008) included 2 control groups gavaged with an equivalent volume of water, one of which was pair-fed the same amount of chow that the EtOH-gavaged dams consumed. The remaining studies did not account for potential additional calories provided by EtOH, instead substituting an equivalent volume of water or saline.

Summary of Reported Outcomes

The majority of included studies reported on outcomes relating to: (i) body composition (specifically percentage body fat) or body mass index (BMI), (ii) glucose metabolism and insulin signaling, and/or (iii) lipid metabolism. Each of these will be summarized in detail below. In addition, there were 4 preclinical studies from 1 laboratory (Zimmerberg, 1989; Zimmerberg et al., 1993a,1993b, 1995) reporting on the potential effects of PAE on brown adipose tissue (BAT) depots. BAT is required for nonshivering thermogenesis, an important mechanism to keep neonates warm (Knobel, 2014). As PAE often results in growth restriction, as well as reduced depots of adipose tissue, this makes neonates with PAE potentially more susceptible to temperature fluctuations. PAE was shown to delay development of thermoregulation in newborn rats by the same laboratory (Zimmerberg et al., 1987), and thermoregulatory deficits were suggested to persist past the third trimester–equivalent period. However, Zimmerberg (1989) found relative BAT weights were not affected by PAE, suggesting that thermoregulatory deficits were not due to reduced substrate availability. The other 3 studies by this group (Zimmerberg et al., 1993a,1993b, 1995) then looked at further aspects controlling the functional status of BAT and found some deficits. However, the quality of reporting in all of these studies was poor (see Fig. 2).

A single study reported on micronutrient metabolism (Kim et al., 2015). This study specifically examined retinoid homeostasis by measuring retinoids in serum and tissues (liver, lung, and prostate) by high-performance liquid chromatography and Western blot. PAE was found to alter retinoid levels in a sex- and tissue-specific manner. Thus, it was hypothesized that some of the effects of maternal alcohol intake on offspring may be due to dysregulation in nutrient (specifically vitamin A) metabolism, rather than a direct effect of alcohol.

Studies Reporting on Body Composition

Outcomes related to body composition are reported in Table 2. Carter and colleagues (2012) used bioelectrical impedance analysis (BIA) to assess percentage body fat and found that children with a FAS or pFAS diagnosis were leaner than nonexposed controls or nonsyndromal children with PAE (Table 2). Similarly, Amos-Kroohs and colleagues (2016) found that their children, predominantly those with FAS/pFAS and males in particular, had a lower BMI than typically developing children. However, when age- and sex-adjusted BMI percentiles were calculated, there was no difference in the percentage that were underweight, normal-weight, or overweight and/or obese between the control and PAE groups. Interestingly, caregivers of children with FASD were more likely to perceive their child as underweight (23% vs. ~1%), which did not match actual measured BMI.

There were five preclinical studies that assessed body composition using dual-energy X-ray absorptiometry (DEXA) and/or magnetic resonance imaging (MRI). Two of these studies challenged offspring with a postnatal HFD, but in both cases, this did not exacerbate the effects of PAE. The low-dose study reported by Probyn and colleagues (2013b) resulted in no effect of PAE on body composition in rats, nor

Table 2. Study Outcomes Related to Body Composition (% Fat Mass)

Study	Postnatal insult "second Hit"	Relevant assessments	Key results[a]	Conclusion
Clinical				
Amos-Kroohs and colleagues (2016)	–	BMI	↓BMI in children with FASD (M only); ↓BMI prepuberty (2 to 10 years); ↔ BMI percentiles	Children with FASD, particularly males, had a lower BMI than typically developing controls
Carter and colleagues (2012)	–	Body fat % by BIA	↓body fat % in children with FAS/pFAS compared to nonexposed and heavy exposed/nonsyndromal	FAS/pFAS diagnoses are associated with a leaner body composition in later childhood
Preclinical				
Periconceptional and early gestation				
Gårdebjer and colleagues (2017)	HFD (from 3 months of age)	Body composition by DEXA	PAE ↑% total body fat mass and % abdominal fat mass (M only)	PAE increased adiposity in adult males. Adiposity increased by HFD but not exacerbated by PAE
Zhang and colleagues (2018)	–	Body composition by DEXA	PAE ↑% fat mass in 12-week-old offspring (M only)	PAE increased adiposity in adult males
Mid–late gestation				
Amos-Kroohs and colleagues (2018)	HFD (from 17 wks for 30 days)	DEXA and MRI	↔% fat mass; ↔% fat mass (M) and ↓% fat mass (F)[b] following HFD	PAE did not increase adiposity. No adiposity risk "unmasked" by a HFD
Throughout gestation				
Dobson and colleagues (2012)	–	MRI of adipose tissue volume	↑visceral and subcutaneous adiposity	PAE results in increased adiposity
Probyn and colleagues (2013b)	–	Body composition by DEXA	↔all body composition measures (including fat mass)	No effect of PAE on body composition

Animal studies are ordered based on the timing of alcohol exposure.

BIA, bioelectrical impedance analysis; BMI, body mass index; DEXA, dual-energy X-ray absorptiometry; F, females; FAS, fetal alcohol syndrome; FASD, fetal alcohol spectrum disorder; HFD, high-fat diet; M, males; MRI, magnetic resonance imaging; PAE, prenatal alcohol exposure; pFAS, partial FAS.

[a]Key results are in alcohol-exposed offspring compared to nonexposed (isocaloric where available) controls.

[b]Compared to the nonexposed water control and isocaloric medium-chain triglyceride control groups.

did one of the studies in mice by Amos-Kroohs and colleagues (2018), when compared against all control groups. Dobson and colleagues (2012) reported PAE-associated adiposity in both males and females in guinea pigs, while Gårdebjer and colleagues (2017) only found this in males using rats, even though exposure was restricted to around conception. Therefore, although the effects of PAE on body composition were variable, heavy PAE resulting in FAS/pFAS produced a tendency for leaner body composition in humans, while heavy exposure in animal models produced a tendency for increased adiposity, particularly in males. Preclinical studies reporting on this domain spanned 3 species, and although the majority of the studies were in the rat, there was evidence for an effect of PAE in both mice and guinea pigs.

Studies Reporting on Glucose Metabolism and Insulin Signaling

Outcomes associated with systemic, physiological glucose metabolism and insulin sensitivity are summarized in Table 3, while molecular and histological outcomes are presented in Table 4. Insulin sensitivity is critical for appropriate glucose removal and storage, and insulin resistance is a component in the development and progression of type 2 diabetes (Kahn et al., 2006). Insulin resistance is also associated with obesity (Hardy et al., 2012; Kahn et al., 2006). Techniques for measurement of glucose tolerance and insulin sensitivity varied across studies and included GTT, insulin tolerance test (ITT), fasting blood glucose and insulin levels, and euglycemic clamp (Table 3). These methods are summarized and compared in a review by Trout and colleagues (2007). Two studies by Yao and colleagues (2006, 2013) conducted a pyruvate challenge, in addition to either an ITT or GTT, to specifically measure gluconeogenesis. Two studies also conducted whole-body metabolic assessments in mice using indirect calorimetry (Amos-Kroohs et al., 2018; Zhang et al., 2018), with only one of these reporting a tendency for PAE to reduce daily energy expenditure in males, which was not statistically significant at the $p < 0.05$ level (Zhang et al., 2018).

Only one clinical study by Castells and colleagues (1981) reported on GTTs performed in children with FAS compared against normal controls in a very small cohort (Table 3). As mentioned above, quality of reporting was poor for this study and no statistics were used to compare the control and FAS groups. Nevertheless, this study reported evidence of high fasting plasma insulin levels (mean ± (SD) standard deviation: FAS: 35 ± 23 μU/ml; control: 6 ± 4 μU/ml) and 3 patients showed elevated

Table 3. Physiological Outcomes Relating to Glucose Metabolism and Insulin Resistance

Study	Postnatal insult "Second Hit"	Relevant assessments	Key results[a]	Conclusion
Clinical				
Castells and colleagues (1981)	–	GTT; serology	↔fasting glucose; ↑fasting insulin; ↑glucose and insulin in some patients following glucose load[b]	Evidence of glucose intolerance and insulin resistance
Preclinical				
Periconceptional and early gestation				
Gårdebjer and colleagues (2015, 2017)	HFD (from 3 months of age)	ipGTT; ipITT; serology	↑fasting glucose; ↑HOMA-IR; ↑AUGC; ↑AUIC; HFD exacerbated IR (M); ↑plasma leptin (F); ↔adiponectin	PAE increased fasting blood glucose. PAE resulted in glucose intolerance and insulin resistance in both sexesExacerbated in males by HFD
Zhang and colleagues (2018)	–	Whole-body metabolic assessment using indirect calorimetry	Trend for ↓VO_2, VCO_2, energy expenditure (M only)[c]; ↔RER (both sexes)	PAE had a tendency to reduce daily energy expenditure in males only
Preconception and throughout gestation				
Lopez-Tejero and colleagues (1989)	–	Oral GTT; serology[d]	↔blood glucose or plasma insulin preweaning; ↑fasting glucose and insulin at PD90 (F only); ↑AUIC and AUGC (PD30); ↑AUIC (PD90)	PAE resulted in insulin resistance in juveniles that persists into adulthood
Ludena and colleagues (1983)	–	Serology[d]	↓glucose and ↑ketone bodies (β-hydroxybutyrate)	PAE altered glucose and fatty acid metabolism
Throughout gestation				
Chen and Nyomba (2003a,2003b)	HFD (at weaning)	ipGTT; serology	↔plasma leptin, adiponectin and nonfasting glucose; ↑nonfasting insulin and resistin; ↑AUIC and AUGC	PAE caused glucose intolerance and hyperinsulinemia, exacerbated by HFD
Chen and Nyomba (2004)	–	IVGTT; IVITT[e]	↑AUGC; ↔AUIC; ↑AIR; ↓K_G; ↓S_I	Impaired glucose tolerance and insulin resistance, regardless of birthweight
Dobson and colleagues (2014)	–	Fasting blood glucose (glucometer)	↔fasting blood glucose	PAE did not change fasting blood glucose
Elton and colleagues (2002)[f]	–	Euglycemic clamp; serology	↔basal glucose and insulin levels[g]; ↔insulin responsiveness[g]	PAE did not affect insulin sensitivity
Probyn and colleagues (2013b)	–	ipGTT; ipITT	↔fasting glucose, insulin, HOMA-IR, AUGC (GTT) or AUIC (GTT); ↑First-phase insulin secretion (M) (GTT); ↓AUGC (F) (ITT)	PAE did not change fasting blood glucose. PAE resulted in sex-specific effects on glucose homeostasis
Yao and colleagues (2006)	–	ipITT[h]; pyruvate challenge	↑fasting insulin and glucose; ↑blood glucose following insulin and pyruvate bolus	PAE increased fasting blood glucose. PAE caused glucose intolerance and insulin resistance
Yao and Nyomba (2007, 2008)	–	ipGTT; euglycemic clamp; pyruvate challenge	↑AUGC and AUIC; ↓insulin-stimulated glucose uptake; ↑blood glucose following pyruvate bolus	PAE resulted in increased gluconeogenesis, insulin resistance, and glucose intolerance
Mid- to late gestation				
Amos-Kroohs and colleagues (2018)	HFD (from 17 wks for 30 days)	Oral GTT, ipGTT, whole-body metabolic assessment using indirect calorimetry	↔fasting glucose/insulin and ↔AUGC[i]; ↔RER or VO_2, even on HFD	PAE did not affect metabolic rate and PAE per se did not alter glucose handling

Continued.

Table 3. (Continued)

Study	Postnatal insult "Second Hit"	Relevant assessments	Key results[a]	Conclusion
He and colleagues (2015)	HFD (at weaning)	Serology	↑fasting glucose for M only when on HFD; ↑fasting glucose (irrespective of diet) and ↑fasting insulin and HOMA-IR only when on HFD (F)	PAE increased fasting blood glucose (F only). PAE resulted in insulin resistance, exacerbated by HFD, in a sex-specific manner (F > M)
Xia and colleagues (2014)	HFD (all animals at weaning) and chronic stress[j]	Serology	↑fasting glucose, ↔fasting insulin, ↑HOMA-IR, only after chronic stress	PAE caused enhanced susceptibility to HFD-induced glucose intolerance and IR, but only following stress
Late gestation				
Yao and colleagues (2014)	–	ipGTT; ipITT	↑AUGC (GTT and ITT), AUIC, HOMA-IR	PAE caused insulin insensitivity and glucose intolerance
Early/mid/late gestation				
Yao and colleagues (2013)	–	ipGTT; pyruvate challenge	↑AUGC and AUIC; ↑blood glucose following pyruvate bolus	PAE caused glucose intolerance and insulin resistance, irrespective of timing of exposure

Preclinical studies are ordered based on the timing of alcohol exposure.

AIR, acute insulin response (AUIC for first 8 minutes after IVGTT); AUGC, area under the glucose curve; AUIC, area under the insulin curve; F, female; GTT, glucose tolerance test; HFD, high-fat diet; HISS, hepatic insulin sensitizing substance; HOMA-IR, homeostatic model assessment of insulin resistance [fasting serum insulin (mU/l) X fasting serum glucose (mmol/l)]/22.5; IHC, immunohistochemistry; ip, intraperitoneal; IR, insulin resistance; ITT, insulin tolerance test; IV, intravenous; K_G, glucose tolerance index (Chen and Nyomba, 2004); M, male; PAE, prenatal alcohol exposure; RER, respiratory exchange ratio [CO_2 production:O_2 consumption]; RIST, rapid insulin sensitivity test; S_I, insulin sensitivity (Chen and Nyomba, 2004); VCO_2, carbon dioxide production; VO_2, oxygen consumption.

[a]Key results are in alcohol-exposed offspring compared to nonexposed (isocaloric where available) controls.

[b]No statistics conducted in this paper and only mean and SD/SEM reported. No AUGC or AUIC calculated for GTT.

[c]*p*-values were 0.10 to 0.16 for these measures.

[d]Enzymatic assay of deproteinized blood.

[e]Insulin injected IV after 8-min blood collection for IVGTT.

[f]Only in vivo outcomes reported.

[g]Irrespective of % fat content in maternal liquid diet.

[h]Glucose and insulin levels were only measured at 2 hours post–ip insulin injection.

[i]Compared to both isocaloric control groups.

[j]From 17 weeks of age for 21 days. Each of the following stressors was administered randomly at an interval of 7 days: (i) food deprivation for 24 hours; (ii) water deprivation for 24 hours; (iii) tail pinch for 5 minutes; (iv) heat stress for 5 minutes; (v) cold swim for 5 minutes; (vi) reversed day/night cycle; and (vii) social isolation for 24 hours. All rats were finally subjected to the cold swim stressor. For more details, see Xia and colleagues (2014).

Table 4. Molecular and Histological Outcomes Relating to Glucose Metabolism and Insulin Resistance

Study	Postnatal insult "Second Hit"	Relevant assessments[a]	Key results[b]	Conclusion
Periconceptional				
Gårdebjer and colleagues (2015, 2017)	HFD (from 3 months of age)	Molecular analysis (qPCR) of liver and visceral adipose tissue	Dysregulated hepatic gluconeogenic genes; altered insulin signaling; ↑leptin and inflammatory cytokine mRNA in adipose tissue	Disruptions to gluconeogenesis and insulin signaling may contribute to the observed PAE-induced glucose intolerance and insulin resistance
Preconception and throughout gestation				
Lopez-Tejero and colleagues (1989)	–	Molecular analysis (enzymatic glycogen assay) of liver	↔liver glycogen (PD15, PD30)	No change in liver glycogen levels in preweanling offspring, despite insulin resistance in adulthood
Ludena and colleagues (1983)	–	Molecular analysis (enzymatic glycogen assay) of liver	↓liver glycogen	Altered glycogen accumulation in the liver
Throughout gestation				
Chen and Nyomba (2003a,2003b)	HFD (at weaning)	Molecular analysis (RT-PCR, WB[c]) of epididymal adipose tissue and muscle; pancreatic histology	↔leptin and adiponectin, ↑resistin mRNA in adipose tissue; ↓GLUT4 in muscle post-ipGTT; ↓ β-cell density and mass only on HFD	Dysregulated resistin (adipose) and glucose (muscle) transport may underlie insulin resistance, with effects exacerbated by a HFD
Chen and colleagues (2005)	–	Molecular analysis (WB[c], insulin binding assay, AKT activity assay) of muscle[d]	↓GLUT4; ↓PI3K activity; ↔AKT activity; disrupted posttranslational modification of insulin receptors	Impaired insulin signaling through the PI3-kinase pathway in skeletal muscle may contribute to insulin resistance
Dobson and colleagues (2012, 2014)	–	Molecular analysis (qPCR) of liver; pancreatic histology and IHC	↓IGF1, IGF1R, IGF2, and ↑IRS2 in a sex-specific manner; ↑pancreatic adipocyte area and ↓% of β-cells in pancreatic islets.	Impaired hepatic insulin/IGF signaling and insulin-producing cells of the pancreas, despite no observed changes to fasting blood glucose
Elton and colleagues (2002)	–	Molecular analysis (WB[c], tyrosine kinase activity, insulin-stimulated glucose uptake) of muscle fibers[e]	↔insulin receptor protein or receptor-associated tyrosine kinase activity[f]; ↓insulin responsiveness (in soleus muscle of M only)[f]	Subtle, sex-specific insulin insensitivity in muscle, but no effect on receptor levels/activity, consistent with normal glucose metabolism
Probyn and colleagues (2013b)	–	Pancreatic histology, molecular analysis (qPCR) of liver, visceral adipose tissue, and muscle	↔β-cell mass/density and islet mass/density; ↔metabolic gene expression (11 genes examined).	No measurable changes in molecular regulators of glucose homeostasis and yet subtle, sex-specific physiological effects reported
Yao and colleagues (2006)	–	Molecular analysis (RT-PCR, WB[c], PEPCK activity) of liver	↑basal and post–insulin bolus PEPCK (mRNA, protein, activity)	Increased expression and activity of gluconeogenic enzymes in offspring exposed to PAE underlies insulin insensitivity and glucose intolerance
Yao and Nyomba (2007, 2008)	–	Molecular analysis (RT-PCR, WB[c]) of muscle and liver	↓GLUT4 and PI3-kinase–mediated insulin signaling (and insulin-stimulated phosphorylation); ↑PTEN and TRB3 (inhibitory)	Dysregulated insulin signaling and glucose transport in muscle and liver underlies PAE-induced insulin resistance
Late gestation				
Yao and colleagues (2014)	–	Molecular analysis (qPCR, WB[c]) of muscle	↓GLUT4 (mRNA and protein); ↑ER stress markers; ↑HDACs	PAE-induced epigenetic changes altered muscle GLUT4 expression and underlies insulin insensitivity and glucose intolerance
Early/mid/late gestation				
Yao and colleagues (2013)	–	Molecular analysis (qPCR, WB[c], ROS/RNS assay, HDAC activity) of liver	↑gluconeogenic enzymes (mRNA and protein); ↑HDAC protein and activity; ↑oxidative and ER stress	Cellular stress and epigenetic modifications underlie PAE-induced gluconeogenesis and glucose intolerance

All studies are preclinical studies that also report on physiological outcomes (see Table 3 for more details). Studies are ordered based on the timing of alcohol exposure.

ER, endoplasmic reticulum; F, female; GLUT4, glucose transporter type 4; HDAC, histone deacetylase; HFD, high-fat diet; IGF, insulin-like growth factor; IHC, immunohistochemistry; M, male; PAE, prenatal alcohol exposure; PD, postnatal day; PEPCK, phosphoenolpyruvate carboxykinase (enzyme involved in gluconeogenesis); PI-3 kinase, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; qPCR, quantitative real-time polymerase chain reaction (for analysis of gene expression); ROS/RNS, reactive oxygen species/reactive nitrogen species (oxidative stress molecules); RT-PCR, reverse transcriptase PCR (product run on a gel and quantified using densitometry); TRB3, tribbles 3 (both PTEN and TRB3 are inhibitors of insulin signaling and glucose transport); WB, Western blot.

[a]Muscle refers to gastrocnemius muscle unless stated otherwise.

[b]Key results are in alcohol-exposed offspring compared to nonexposed (isocaloric where available) controls.

[c]WB gels not shown with ladder and/or loading control or not shown in paper at all.

[d]Five minutes post–IV insulin injection.

[e]Red (soleus) and white (extensor digitorum) muscle fibers.

[f]Irrespective of % fat content in maternal liquid diet.

plasma glucose (FAS: 186 to 210 mg/ml; control: 135 mg/ml) and insulin (FAS: 180 to 300 μU/ml; control: 51 μU/ml) levels 0.5 hours post–glucose load in a GTT. These results suggest some degree of glucose intolerance and insulin resistance in these patients with FAS.

There were 18 preclinical studies reporting on glucose metabolism and insulin resistance (Table 3). These varied in timing of alcohol exposure across gestation, but PAE resulted in adverse outcomes irrespective of timing. All but 3 of these studies reported glucose intolerance and/or insulin resistance in offspring with PAE, exacerbated by a postnatal HFD where this was given as a "second Hit" The study with the lowest EtOH exposure, Probyn and colleagues (2013b), reported a very subtle phenotype, with increased first-phase insulin secretion in males only following a GTT and decreased area under the glucose curve (AUGC) in females only following an ITT. For the 3 studies showing no phenotype, one reported that fasting blood glucose remained similar between offspring with PAE and controls, as measured by glucometer in guinea pigs (Dobson et al., 2014), one assessed insulin sensitivity by euglycemic clamp in rats (Elton et al., 2002), and one compared PAE against 3 control groups (2 isocaloric and 1 negative caloric/water) and found that PAE *per se* did not alter glucose handling using a mouse model (Amos-Kroohs et al., 2018; Table 3).

To examine molecular mechanisms potentially underlying the observed glucose intolerance and insulin resistance, studies used a variety of assessment techniques including quantitative polymerase chain reaction (PCR) to examine gene expression, Western blot to measure protein content, enzymatic assays to measure glycogen levels, and various activity assays (e.g., AKT, tyrosine kinase, phosphoenolpyruvate carboxykinase [PEPCK]; Table 4). These studies were conducted using liver, gastrocnemius muscle, and adipose tissue. Disruptions to gluconeogenesis, glucose transport, IGF signaling, and/or insulin signaling pathways were identified in these tissues (Table 4). One study also reported PAE-induced epigenetic changes to glucose transporter expression in muscle tissue (Yao et al., 2014). In addition, 3 studies used pancreatic histology/immunohistochemistry to examine β-cell density (Table 4). Only one of these studies showed reduced β-cells using a guinea pig model (Dobson et al., 2012), suggesting that in most cases, disruption to insulin signaling rather than insulin production underlies altered glucose homeostasis in response to PAE.

Studies Reporting on Lipid Metabolism

Outcomes related to altered lipid metabolism are reported in Table 5, with many studies reporting hypercholesterolemia and/or dyslipidemia in offspring with PAE, irrespective of the timing of EtOH exposure during gestation. Only preclinical studies investigated this domain in response to PAE, all conducted in the rat. Most of these studies also reported glucose intolerance and/or insulin resistance (see Table 3), suggesting that PAE is often associated with a full metabolic syndrome in offspring. Assessments used to examine lipid metabolism were primarily serology to measure systemic triglycerides (TG), total cholesterol (TCH), low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL), and molecular or histological examination of the liver (Table 5). The only study that did not report a phenotype, the low-dose chronic model by Probyn and colleagues (2013b), measured plasma TG only. This study also reported only a subtle, sex-specific effect on glucose homeostasis (Table 3), suggesting that dose rather than timing may be important in development of dyslipidemia in offspring. There also appeared to be a sex-specific effect, although 2 studies reported stronger phenotypes in males (Gårdebjer et al., 2017; Pennington et al., 2002) while one study saw a more pronounced effect in females (Qi et al., 2017; Table 5). Specific examination of the liver revealed PAE-induced micro- and macrovesicular steatosis, increased TG concentration, and dysregulated cholesterol-metabolizing genes (Table 5). Most studies in this group included a postnatal "second Hit" in their experimental design (HFD with or without stress), and in all cases, this either unmasked adverse effects by PAE or exacerbated the phenotype (Table 5). Interestingly, the study by Ni and colleagues (2015) showed that PAE resulted in a concomitant enhanced susceptibility to HFD-induced osteoarthritis with the observed hypercholesterolemia.

DISCUSSION

Although neurological, cognitive, and behavioral deficits of children prenatally exposed to alcohol are well characterized, there has been growing interest in defining the complete range of associated adverse health outcomes. In particular, evidence for increased risk of developing chronic conditions such as type 2 diabetes, cardiovascular disease, and obesity would allow early, appropriate interventions to improve long-term health and well-being. This systematic review has uncovered a broad range of PAE-induced health outcomes, covering all major body systems, and suggests that maternal alcohol consumption can play a role in DOHaD. This includes impacts on the cardiorenal system (N Reid, LK Akison, W Hoy, KM Moritz, unpublished data); male and female reproductive systems (Akison et al., 2019); the immune system and susceptibility to infection and atopic allergy (N Reid, KM Moritz, LK Akison, unpublished data); digestive system and liver function; and glucose and lipid metabolism, which is the focus of this review. However, the vast majority of studies used preclinical models (>80%), highlighting an important gap in our knowledge of health outcomes in clinical cohorts with PAE. Given the recent conservative estimate that up to 5% of children in the United States may have FASD (May et al., 2018), our results suggest future studies into FASD comorbidities are urgently required.

Studies specifically investigating metabolic outcomes in offspring with PAE are relatively recent, with 50% published in the last 10 years and greater than 75% within the last

Table 5. Study Outcomes Investigating Lipid Metabolism/Dyslipidemia

Study	Postnatal insult "Second Hit"	Relevant assessments[a]	Key results[b]	Conclusion
Periconceptional				
Gårdebjer and colleagues (2017)	HFD (from 3 months of age)	Serology (TG, LDL, HDL, THC); liver histology	↑TG, HDL, and THC (M); ↑LDL, THC (F); ↑expression of TNF-α and IL-6 in adipose tissues (M); ↑microvesicular liver steatosis (M)	PAE increased plasma TG. PAE altered plasma lipid profiles and induced liver steatosis in a sex-specific manner, similar to a HFD
Throughout gestation				
Chen and Nyomba (2004)	–	Serology (FFA, TG); tissue TG (muscle, liver)	↔Plasma FFA; ↑TG in plasma, muscle, liver in IUGR offspring only	PAE increased plasma TG. PAE-induced IUGR results in dyslipidemia.
Pennington and colleagues (2002)	Age (→PD360); ↓ testosterone by castration (M)	Serology (TG, LDL, VLDL, HDL)	↑TG, exacerbated by age (M) and maternal stress[c] (M/F); ↑VLDL; castration prevented elevated TG (M)	PAE increased plasma TG (M). Exacerbated by age and maternal stress
Probyn and colleagues (2013b)	–	Serology (TG)	Trend for ↓TG in M; ↔TG in females	No change to plasma TG
Mid- to late gestation				
He and colleagues (2015)	HFD (at weaning)	Serology (TG, TCH)	↑TG and TCH, mainly in offspring exposed to HFD	PAE enhanced susceptibility to elevated TG and TCH induced by HFD
Ni and colleagues (2015)	HFD (all animals at weaning)	Serology (TCH, LDL, and HDL)	↑TCH and LDL; ↓HDL (all offspring on HFD)	PAE increased TCH in response to a HFD.
Qi and colleagues (2017)	HFD (at weaning)	Serology (TCH, LDL, HDL); molecular analysis (qPCR) of liver	↑TCH and LDL, ↓HDL (F > M); dysregulated cholesterol-metabolizing genes; HFD enhanced PAE effects	PAE increased TCH, exacerbated by HFD, with effects more pronounced in F than M
Xia and colleagues (2014)	HFD (all animals at weaning) and chronic stress[d]	Serology (TG, TCH, LDL, HDL); liver histology	↑TCH, LDL, and ↓HDL both basal and poststress; hepatic macrovesicular steatosis	PAE increased TCH in response to a HFD

All studies are preclinical and are ordered based on the timing of alcohol exposure.

EtOH, ethanol; F, female; FFA, free fatty acids; HDL, high-density lipoprotein cholesterol; HFD, high-fat diet; IUGR, intrauterine growth restriction; LDL, low-density lipoprotein cholesterol; M, male; PAE, prenatal alcohol exposure; PD, postnatal day; qPCR, quantitative real-time polymerase chain reaction (for analysis of gene expression); T, testosterone; TCH, total cholesterol; TG, triglycerides; VLDL, very low–density lipoprotein cholesterol.

[a]Muscle refers to gastrocnemius muscle unless stated otherwise.

[b]Key results are in alcohol-exposed offspring compared to nonexposed (isocaloric where available) controls.

[c]Maternal stress was a restraint stress used during blood sampling.

[d]From 17 weeks of age for 21 days. Each of the following stressors was administered randomly at an interval of 7 days: (i) food deprivation for 24 hours; (ii) water deprivation for 24 hours; (iii) tail pinch for 5 minutes; (iv) heat stress for 5 minutes; (v) cold swim for 5 minutes; (vi) reversed day/night cycle; and (vii) social isolation for 24 hours. All rats were finally subjected to the cold swim stressor. For more details, see Xia and colleagues (2014).

20 years, when compared with studies focused on neurological and behavioral outcomes, which date back to the early 1970s (Jones and Smith, 1973). However, only 3 clinical studies to date met all inclusion/exclusion criteria for this review. This highlights the challenge of obtaining data from cohorts with appropriate nonexposed control participants. Due to the paucity of studies in this area, we included the study by Carter and colleagues (2012), even though the control group did potentially have some alcohol exposure. Given the high rates of alcohol consumption in the general population of reproductive-age women, with recent estimates in countries such as Australia (Australian Institute of Health and Wellbeing, 2013) and the United States (Robbins et al., 2014) around 50 to 80%, and the current rates of unplanned pregnancies (~50%; Colvin et al., 2007), we argue that it will often be difficult to obtain all control participants with guaranteed zero PAE.

One study that we did exclude from our metabolic/body composition analysis by Fuglestad and colleagues (2014) used a no-FASD control group, recruited from patients referred to a FASD assessment clinic due to known or suspected PAE. Given these patients were exposed but nonsyndromal, this study was outside the scope of this review. They argued that the no-FASD group was a better reference group than using a traditional "healthy" nonexposed control group to better control for potential confounders (e.g., socioeconomic status, race, family characteristics). Interestingly, they compared the prevalence of overweight/obesity in their participants with FASD against national and state prevalence rates and their no-FASD group. They found that children with FAS tended to be underweight while children with pFAS and adolescent females with FASD tended to be overweight/obese (Fuglestad et al., 2014). Similarly, earlier studies provide data from cohorts with FASD to confirm this dichotomy in BMI (Klug et al., 2003; Spohr et al., 2007; Werts et al., 2014). The clinical studies included in this review support a lower BMI/body fat percentage in young children with FAS/FASD, and Amos-Kroohs and colleagues (2016) also had data to suggest that postpuberty, BMI may increase more with age in children with FASD. However, the latter study did not separate the analysis by diagnosis (i.e., FAS vs. pFAS). However, Carter and colleagues (2012) also reported a lower body fat percentage in children with pFAS, although they only examined children at 9 years of age. It should be noted that these studies report on very different study populations, one reporting a South African cohort with very low socioeconomic status (Carter et al., 2012) and the other being a case–control study from the United States (Amos-Kroohs et al., 2016), and therefore, postnatal factors such as diet would be very different. Therefore, there is an urgent need for accurate assessment of BMI in children with FASD, particularly into adolescence, to reduce long-term adverse health outcomes associated with overweight/obesity.

Amos-Kroohs and colleagues (2016) proposed that the increased adiposity risk in children with FASD may originate from behavioral and/or dietary practices. One other study reported that children with FASD have disrupted eating behaviors (Werts et al., 2014), as did the clinical and preclinical studies by Amos Kroohs and colleagues that were included in this review. However, the latter studies reported little to no effect on body composition or BMI. Additionally, a recent preclinical study using a rat model has shown that adult male offspring with PAE show an increased preference for a HFD (Dorey et al., 2018). Consequently, further preclinical and clinical research is needed that considers both behavioral and/or dietary practices and metabolic outcomes in relation to PAE.

Only one other clinical study was found using our search criteria, but had poor quality of reporting and was published >30 years ago. It suggests that the glucose intolerance and insulin resistance measured in offspring with PAE from preclinical studies may also occur in clinical cohorts, at least where alcohol exposure is severe enough to result in a FAS diagnosis. Given the evidence discussed above for altered body composition in children with FAS, and the role that both skeletal muscle and adipose tissue play in insulin signaling and glucose homeostasis, changes in body composition are important for glucose clearance. However, to date, no clinical studies have looked at body composition, both percentage fat mass and muscle mass, in conjunction with physiological measurement of glucose handling in individuals with FAS and compared against nonexposed controls. Only 4 preclinical studies in this review included measurements of both of these outcomes but showed inconsistent results, and only 2 specifically measured muscle mass. Therefore, we recommend that future clinical studies include comprehensive assessment of body composition, in addition to measurement of glucose handling, in individuals with FAS and FASD as well as normally developing, nonexposed controls. Whether children without more severe FAS but still diagnosed with FASD also show glucose intolerance and insulin resistance, indicative of a diabetic phenotype, remains to be determined. We suspect that the risk of developing type 2 diabetes later in life will be higher in children with FASD, but a longitudinal approach will be required for future research in this area.

The preclinical studies included in the current review provide evidence of glucose intolerance, insulin resistance, dyslipidemia, hypercholesterolemia, and/or increased percentage body fat associated with PAE, although specific outcomes varied across studies and often the phenotype only emerged when animals were challenged (e.g., with a postnatal HFD). While the majority of the studies were conducted in the rat, there was evidence for effects of PAE in mouse and guinea pig, suggesting some generalization across rodent species. However, given that both mouse studies used a shorter exposure either early (Zhang et al., 2018) or later in gestation (Amos-Kroohs et al., 2018), direct comparison with rat studies with exposure throughout gestation is problematic. The use of outbred pairings in DOHaD research has been recommended as a better mimic for natural outbred populations, such as humans (Dickinson et al., 2016).

Deleterious outcomes occurred irrespective of the timing of alcohol exposure during gestation, but the severity of the

phenotype appeared to be related to dose. Most studies used moderate-to-high doses of alcohol (3 to 4 g/kg/d). Only one study by Probyn and colleagues (2013b) used a chronic, low dose of alcohol that resulted in a peak BAC of only 0.03%. This study examined glucose and lipid metabolism, as well as body composition, and only reported a subtle, sex-specific effect on glucose homeostasis. The fact that any phenotype at all could be detected is a cause for concern and supports health authority guidelines that no level of alcohol consumption is the safest option when pregnant or planning a pregnancy (National Health and Medical Research Council, 2009; World Health Organization, 2004).

Preclinical models covered a wide range of timings of alcohol exposure during gestation. Yao and colleagues (2013) restricted exposure to early (up to day 7), mid (days 8 to 14)-, or late (day 15 to birth) gestation and found that all resulted in disrupted gluconeogenesis, and oxidative and endoplasmic reticulum (ER) stress. Interestingly, pups from the early-exposure group were the only group not growth restricted. Only 2 studies by Gårdebjer and colleagues restricted alcohol exposure to the periconceptional period, including one estrous cycle prior to mating (Gårdebjer et al., 2015, 2017). This is a common scenario for PAE in unplanned pregnancies, where alcohol consumption typically ceases following pregnancy recognition (McCormack et al., 2017). This short window of high alcohol exposure (~0.18 to 0.25% BAC) resulted in glucose intolerance, insulin resistance, dyslipidemia, increased adiposity, and liver steatosis in adult offspring. This is perhaps surprising, given that the fetal organs were not formed at the time of exposure. To explore epigenetic changes of the early embryo as a possible mechanism, the authors measured epigenetic markers in fetal livers at late gestation and found increased expression of DNA methyltransferases in offspring with PAE, potentially resulting in a predisposition to metabolic dysfunction in later life (Gårdebjer et al., 2015). This highlights the importance of the pre- and periconceptional maternal environment to set a healthy trajectory for future offspring. Overall, the preclinical studies suggest that alcohol exposure at all stages of pregnancy has the potential to result in metabolic dysfunction, although the use of different animal models and doses of alcohol makes it difficult to establish the most vulnerable window of exposure.

Although PAE is often associated with growth restriction in offspring, this is not always the case (O'Leary et al., 2009). Among the included preclinical studies, 6 reported no differences in birthweight between offspring with PAE and controls, while a further 2 studies did not report on birthweights (see Table 1). Although growth restriction resulting in small-for-gestational-age (SGA) offspring has been associated with an increased risk for metabolic syndrome in adulthood, it is not a prerequisite (Euser et al., 2010; Kopec et al., 2017). All but 2 of the 8 preclinical studies with no evidence of SGA still reported impairments in offspring with PAE, irrespective of birthweight. Interestingly, the Canadian guidelines for diagnosis of FASD, which are also followed in Australia and New Zealand, were revised in 2015 to remove growth deficits as an essential criterion for diagnosis (Cook et al., 2016), although this has been regarded as a controversial change by others (Astley et al., 2016).

Most preclinical studies attempted to allow for potential differences in diet consumption rates or EDC in their nonexposed control groups. This is important, as calorie restriction or excess is known to program metabolic outcomes in offspring (Langley-Evans, 2006) and could mask any specific actions of alcohol. Alcohol itself is a source of calories, providing 7 kilocalories/gram. However, whether the commonly used substitutes for additional calories provided by EtOH are the most appropriate type of calories is debatable. The most recent study by Amos-Kroohs and colleagues (2018) makes this point and highlights the importance of appropriate controls. They found that PAE-specific effects on offspring glucose tolerance and adiposity only emerged when offspring with PAE were compared against water-only or maltodextrin isocaloric controls, common controls used by other studies. However, there were no effects of PAE *per se* when compared against offspring fed an isocaloric medium-chain triglyceride diet. The authors argued that this was a more metabolically equivalent control for alcohol than a carbohydrate-based control and concluded that prior reports of metabolic dysfunction in adult offspring with PAE reflect added gestational calories and not a pharmacological action of alcohol. However, it is important to note that this study was one of the few mouse studies and only treated with alcohol for 6 days from GD12.5 to GD17.5, the shortest period of alcohol exposure in the current review. Given this is the first and only study to date that has used this novel isocaloric control, further research is needed, including across other models of alcohol exposure.

Interestingly, Yao and colleagues (2013, 2014) reported on the use of tauroursodeoxycholic acid (TUDCA), a putative neuroprotective bile acid, to reverse epigenetic changes and dysregulation of gluconeogenic proteins caused by PAE in both males and females. TUDCA successfully reversed these effects, as well as normalizing glucose and insulin levels following an ipGTT/ipITT, demonstrating the potential to be used as a therapeutic to treat glucose intolerance associated with PAE. TUDCA has also been used in at least one clinical study to treat insulin resistance (Kars et al., 2010).

CONCLUSION

The current review suggests that PAE may affect a broad array of organs and systems of the body and contribute to a range of long-term health outcomes. This includes impacts on metabolism, cardiorenal function, reproductive outcomes, and the immune system in offspring. In particular, this review provides evidence from preclinical studies that PAE may contribute to dysregulation of glucose homeostasis, which has long-term implications for the development of type 2 diabetes in individuals with PAE. However, the scarcity of clinical studies is particularly concerning and future

studies on cohorts examining metabolic health of children and young adults with FAS/FASD are urgently required. We suggest individuals with PAE are potentially at an increased risk of developing type 2 diabetes and/or metabolic syndrome as they age and thus require appropriate clinical advice and monitoring.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Summary of studies from other health domains not described in detail in this review. See Fig. for further details of domains.

Data S2. Quality assessment of included clinical and preclinical studies.