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See corresponding commentary on page 1099.

Replacing Part of Glucose with Galactose in the Postweaning Diet Protects Female But Not Male Mice from High-Fat Diet–Induced Adiposity in Later Life

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ABSTRACT

Background: Duration of breastfeeding is positively associated with decreased adiposity and increased metabolic health in later life, which might be related to galactose.

Objective: The aim of this study was to investigate if partial replacement of glucose with galactose in the postweaning diet had a metabolic programming effect.

Methods: Male and female mice (C57BL/6JRccHsd) received an isocaloric diet (16 energy% fat; 64 energy% carbohydrates; 20 energy% protein) with either glucose (32 energy%) (GLU) or glucose + galactose (GLU + GAL, 16 energy% each) for 3 wk postweaning. Afterwards, all mice were switched to the same 40 energy% high-fat diet (HFD) for 9 wk to evaluate potential programming effects in an obesogenic environment. Data were analyzed within sex.

Results: Female body weight (−14%) and fat mass (−47%) were significantly lower at the end of the HFD period (both $P < 0.001$) among those fed GLU + GAL than among those fed GLU; effects in males were in line with these findings but nonsignificant. Food intake was affected in GLU + GAL–fed females (+8% on postweaning diet, −9% on HFD) compared with GLU-fed females, but not for hypothalamic transcript levels at endpoint. Also, in GLU + GAL–fed females, serum insulin concentrations (−48%, $P < 0.05$) and the associated homeostasis model assessment of insulin resistance (HOMA-IR) were significantly lower ($P < 0.05$) at endpoint, but there were no changes in pancreas morphology. In GLU + GAL–fed females, expression of insulin receptor substrate 2 (*Irs2*) (−27%, $P < 0.01$; −44%, $P < 0.001$) and the adipocyte size markers leptin (*Lep*) (−40%, $P < 0.05$; −63%, $P < 0.05$) and mesoderm-specific transcript homolog protein (*Mest*) (−80%, $P < 0.05$; −72%, $P < 0.05$) was lower in gonadal and subcutaneous white adipose tissue (WAT), respectively. Expression of insulin receptor substrate1 (*Irs1*) (−24%, $P < 0.05$) was only lower in subcutaneous WAT in GLU + GAL–fed females.

Conclusions: Partial replacement of glucose with galactose, resulting in a 1:1 ratio mimicking lactose, in a 3-wk postweaning diet lowered body weight, adiposity, HOMA-IR, and expression of WAT insulin signaling in HFD-challenged female mice in later life. This suggests that prolonged galactose intake may improve metabolic and overall health in later life. *J Nutr* 2019;149:1140–1148.

Keywords: galactose, lactose, programming, adipose tissue, insulin signaling, postweaning

Introduction

The quality of the early-life diet can have lasting effects, ameliorating glucose and lipid metabolism as well as attenuating adiposity and obesity, provided this nutritional exposure occurs during a critical window of development. This phenomenon was reported first by Barker (1), who hypothesized that fetal undernutrition affects morbidity and mortality in adulthood. Later, data from, in particular, the Dutch Famine Cohort showed that early-life undernutrition increased later-life susceptibility to diseases like type 2 diabetes (2). Since then, a substantial body of literature has supported the impact of the maternal environment on the phenotype of the offspring (3). Whereas effects of under- and overnutrition during pregnancy as well as nursing on later-life health are well established, the impact of early-life nutrition (weaning and early postweaning) has been studied less, although

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animal studies have shown lasting effects on later-life metabolic health (4–7).

Weaning is characterized by a change in nutritional profile (8) and diversification from lactose as the primary carbohydrate source to a variety of sugars and polysaccharides (9). In humans, timing of first weaning foods, types of weaning foods—which may include fruits and vegetables, dairy, and cereals—and duration of weaning all vary according to cultural and individual preferences (10). As a result, there is great variability among individuals in the types and amounts of carbohydrates to which they are exposed in late infancy and early childhood (during and shortly after weaning).

Lactose, a disaccharide of glucose and galactose, is the main carbohydrate in breast milk, providing ~44% of its energy (11); i.e., ~22% of the total energy comes from glucose and ~22% from galactose. The relative contribution of carbohydrates to total energy intake in young children (2–3 y) in the Netherlands increases during the postweaning phase to ~58% (12), but decreases again to ~48% in adulthood [19–30 y (13)]. In the postweaning diet, energy from carbohydrates comes mainly from glucose, in the form of simple sugars and a variety of (complex) polysaccharides. Fructose also makes a sizable contribution to energy intake in postweaning diets [9% of the energy; 7–69 y (14)], whereas galactose barely contributes. Current nutritional guidelines are not specific about the type of carbohydrate to give during the weaning and (early) postweaning periods, although products containing added sugar, fruit juices, and sugar-sweetened beverages are discouraged (15). However, because the early postweaning period covers a critical period of development that is amenable to nutritional programming, it is important to elucidate the differential effects of carbohydrates and to clarify to what extent carbohydrates can affect long-term (adult) health.

Extended breastfeeding (>12 mo of age) is associated with protection from obesity and data suggest the effect is due to milk constituents rather than other environmental factors (16–18). Because lactose is the main energy source during breastfeeding, it is tempting to speculate that high intakes of lactose might explain, at least in part, these health benefits. Further, half of the monosaccharides derived from lactose are galactose, a monosaccharide that, otherwise, is hardly present in the diet of older infants and young children.

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Supplemental Tables 1–3, Supplemental Methods, and Supplemental Figures 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
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Abbreviations used: *Acacb*, acetyl-CoA carboxylase 2; *Acadl*, acyl-CoA dehydrogenase; *Agrp*, agouti-related peptide; *B2m*, Beta-2 microglobulin; BC, body composition; BW, body weight; *Canx*, Calnexin; en%, energy percent; *Fabp4*, fatty acid binding protein 4; FI, food intake; FM, fat mass; GLU, diet with 32 en% glucose; GLU + GAL, diet with 16 en% galactose and 16 en% glucose; gWAT, gonadal white adipose tissue; HFD, high-fat diet; *Insr*, insulin receptor; *Irs*, insulin receptor substrate; *Lep*, leptin; *Lipe*, hormone-sensitive lipase; *Leprb*, leptin receptor long isoform; LM, lean mass; *Mest*, mesoderm-specific transcript homolog protein; *Npy*, neuropeptide Y; OGTT, oral-glucose-tolerance test; *Pck1*, phosphoenolpyruvate carboxykinase 1; *Plin1, perilipin 1*; *Pnpla2*, patatin like phospholipase domain containing 2; *Pomc*, pro-opiomelanocortin; *Rplp0*, Ribosomal protein large P0; *Rps15*, Ribosomal protein S15; *Socs3*, suppressor of cytokine signaling 3; sWAT, subcutaneous white adipose tissue; TG, triglyceride; WAT, white adipose tissue.

Currently, there is some evidence for nutritional programming by carbohydrates, which focuses on pregnancy or the nursing period (for various parameters). A high-carbohydrate formula given to rats during suckling caused lasting alterations in hypothalamic gene expression, which led to hyperphagia, greater body weight (BW) gain, and higher circulating leptin concentrations—a marker of higher fat—in adulthood (19). Similarly, sucrose feeding during pregnancy and lactation led to increased hepatic triglyceride (TG) content and lower insulin and glucose tolerance in rat offspring (20). Overall, fructose intake during pregnancy and lactation increased the chance of metabolic disturbances in the hepatic and adipose tissue of offspring [reviewed in (21)]. To our knowledge, nutritional programming studies with galactose are lacking, thus far.

In the present study, we aimed to establish whether galactose can affect long-term metabolic health: more specifically, whether partial replacement of glucose by galactose in the postweaning diet, mimicking lactose, affects body composition (BC) or metabolic profiles including ectopic lipid storage, using a mouse model of nutritional programming. To assess this, C57BL/6JRccHsd mice were given a starch-based diet containing additional monosaccharides at 32 energy percent (en%) from glucose, or 16 en% glucose + 16 en% galactose to mimic lactose intake, for 3 wk postweaning before being switched to a galactose-free high-fat diet (HFD) for 9 wk. We hypothesized that including galactose in the postweaning diet would protect the mice from adiposity in later life. We, therefore, mainly focused our molecular analyses on adipose tissue, but also studied liver TG content, pancreas morphology, and hypothalamic gene expression. White adipose tissue (WAT) metabolic pathways were examined in more detail.

Methods

Ethical approval

All experimental procedures were approved by the Animal Experimental Committee (DEC 2014085, Wageningen, Netherlands) and were carried out in accordance with the principles of good laboratory animal care, following the European Union Directive for the protection of animals used for scientific purposes (86/609/EEC). This experiment was included as part of a larger study investigating the nutritional programming effects of different monosaccharides during the postweaning period, reducing the number of experimental animals used, in line with the 3R principles. More specifically, the glucose control group were also used as controls for an experiment investigating postweaning programming effects of fructose. Data from this control glucose group [i.e., BW, food intake (FI), fat mass (FM), lean mass (LM), organ weights, glucose tolerance, circulating leptin concentrations, serum insulin concentrations, and hepatic TG content] have been reported previously (22).

Diets

Diets were ordered from Research Diet Services BV. The compositions of the breeding and postweaning diets were in accordance with the AIN-93 guidelines for growing rodents (23); see **Supplemental Table 1** for a complete overview of the compositions. The postweaning diets differed only in monosaccharide composition: we compared a glucose-only diet (GLU) with one containing a 1:1 mixture of glucose and galactose (GLU + GAL) mimicking lactose. A 1:1 mixture was used rather than lactose to prevent problems associated with lactose intolerance, due to lactase deficiency, in mice after weaning. Both postweaning diets had 32 en% from monosaccharides (i.e., 32 en% glucose in GLU or 16 en% galactose and 16 en% glucose in GLU + GAL) and contained 16 en% fat, 20 en% protein, and 32 en% starch, as well as mineral and vitamin mixes compliant with the AIN-93 guidelines (23).

After the 3-wk postweaning period, all mice were switched to the same HFD with 40 en% fat, 20 en% protein, and 40 en% carbohydrates (starch and sucrose) as well as mineral and vitamin mixes (Supplemental Table 1). The HFD corresponded to the human macronutrient intake profile and induced adiposity (24, 25).

Animals, study design, and measurements

Male and female C57BL/6JRccHsd mice were obtained from Harlan (Harlan Laboratories BV). C57BL/6JRccHsd mice are an established model for diet-induced obesity and, as do humans, have an intact nicotinamide nucleotide transhydrogenase gene, which is absent in many BL/6J strains. The mice were given a semisynthetic breeding diet (Research Diet Services; 16 en% fat, 20 en% protein, 64 en% carbohydrates, see Supplemental Table 1) and time-mated. Nests were standardized to 6 pups per nest 0–2 d after birth, with 2–4 female pups per nest. Female and male pups were included in the experiments. After weaning at 3 wk of age, the pups were stratified by sex and BW, maximizing the number of nests contributing to the dietary groups, and given either the GLU postweaning diet or the GLU + GAL postweaning diet. After the 3-wk postweaning intervention, all the animals were switched to the same HFD for 9 wk. Ultimately, males were $n = 12$ per group, whereas GLU-fed females were $n = 14$ and GLU + GAL–fed females were $n = 13$. BW and FI were measured weekly. Energy intake was calculated by multiplying FI (in grams) by diet energy content (Supplemental Table 1). BC was determined (without anesthesia) using an EchoMRI 100 V (EchoMedical Systems) (26) and measured weekly during the postweaning period and biweekly during the HFD period. Food and water were available ad libitum. During all procedures, the mice were kept in a controlled environment (23°C ± 1°C; 12 h:12 h light/dark cycle).

Oral-glucose-tolerance test

During week 11, an oral-glucose-tolerance test (OGTT) was performed, as described previously (22).

Organ collection and processing

At the end of the HFD feeding period, the animals were deprived of food for 2–5.5 h from the start of the light phase, before being killed; decapitation was in a random order. Blood glucose concentrations were measured using a standardized method (Freestyle). MiniCollect serum tubes (Greiner Bio-One BV) were used to collect serum. Livers were collected, weighed, and snap-frozen in liquid nitrogen. Gonadal WAT (gWAT) was excised; left fat pads were snap-frozen in liquid nitrogen, whereas right fat pads were weighed and fixed in paraformaldehyde. Subcutaneous WAT (sWAT) was collected from the inguinal region and snap-frozen in liquid nitrogen. Pancreatic tissue was separated from mesenteric WAT, based on density in PBS; both organs were weighed and pancreatic tissue was fixated in paraformaldehyde. Hypothalami were collected and snap-frozen in liquid nitrogen. All samples were stored at −80°C until analysis.

Serum measurements

Serum leptin was measured using Bio-Plex Pro mouse diabetes assays (Bio-Rad Laboratories) in accordance with the manufacturer's instructions. Serum insulin was measured using the Ultra-Sensitive Mouse Insulin ELISA Kit (ChrystalChem) following the manufacturer's instructions. Serum measurements were done in duplicate. HOMA-IR was calculated from serum insulin (in milliunits per liter.) and whole blood glucose (in millimoles per liter) using a factor of 14.1, which is applicable for C57BL/6J mice (27).

TG extraction and measurement

Hepatic TG content was measured using the Liquicolor kit (HUMAN) and expressed per protein content, as published previously (22).

Pancreatic β-cell mass and β-cell area

Analysis of pancreatic β-cell mass and area was performed as published elsewhere (22), with the modification that β-cell area was determined using Adobe Photoshop.

Analysis of adipocyte size gWAT

Adipocyte size was determined using images of Mayer's haematoxylin-stained gWAT; details can be found in the **Supplemental Methods**.

RNA extraction

Total RNA was extracted from hypothalami, gWAT, and sWAT. Tissues were ground with mortar and pestle in liquid nitrogen before being dissolved in TRIzol reagent (Invitrogen). After TRIzol extraction, additional chloroform, phenol/chloroform/isoamyl-alcohol, and chloroform extraction steps were performed. Total RNA was precipitated with isopropanol, washed with ethanol, and dissolved in DNase/RNase-free water. RNA concentrations were measured using a Nanodrop spectrophotometer (IsoGen Life Science). RNA quality was checked using an Experion automated electrophoresis system (Bio-Rad). In addition, sWAT RNA quality was checked on an Agilent 2200 Tapestation (Agilent Technologies Inc.) with RNA ScreenTape (Agilent). Subsequently, RNA was converted to cDNA using an iScript kit (Bio-Rad) according to the manufacturer's instructions (input: 1 μg RNA for gWAT and sWAT, 22.5 ng RNA for hypothalamus).

Quantitative RT-PCR

Transcript expression was measured using iQ SYBR Green Supermix (Bio-Rad) on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad), with 3 min at 95°C, 40 cycles of 15 s at 95°C and 45 s at the annealing and elongation temperature, followed by melt-curve analysis. Primers were designed using NCBI Primer-Blast (NCBI website); an overview of primer sequences and annealing temperatures is given in **Supplemental Table 2**. cDNA from all samples was pooled and serial dilutions were used for standard curves; for each transcript, 2 negative controls were also included (water and a sample without reverse transcriptase). Samples were measured in a 100-fold dilution; samples and standards were measured in duplicate. Low-level expressed genes were preamplified with SsoAdvanced PreAmp Supermix (Bio-Rad) to ensure good technical results. For gWAT, preamplification consisted of 3 min at 95°C, followed by 10 cycles of 15 s at 95°C and 45 s at 58°C. For sWAT, preamplification was performed in 100-fold diluted cDNA for 15 cycles (genes that were preamplified are listed in Supplemental Table 2). Preamplification of hypothalamic samples, carried out for all target and reference genes, was for 16 cycles. A cDNA pool of the preamplified samples was made for the standard curves and quantitative RT-PCR was carried out as described above. Negative controls were included in the preamplification. Stable gene expression levels were determined using CFX Manager software (version 3.1; Bio-Rad), and data normalized against stable reference genes, namely Ribosomal protein large P0 *(Rplp0)*, Calnexin *(Canx)*, Beta-2 microglobulin (*B2m*), and Ribosomal protein S15 (*Rps15)* for gWAT; *Rplp0*, *B2m*, and *Rps15* for sWAT; and *B2m* and *Rps15* for hypothalami. Data were normalized to expression in the GLU-fed group, which was set to 1 for each gene.

Statistics

Data were analyzed within sex, for each sex separately, because many physiological parameters differ between the sexes, and programming effects are often sex-dependent. Statistical analyses were performed in GraphPad Prism version 5.04 (GraphPad Software Inc.). Two-factor repeated-measures ANOVA was used for analysis of BW, FM, and LM, with postweaning diet as the between-subject factor, time as the within-subject factor, and group × time interaction. The postweaning and HFD periods were studied separately. When the effects of postweaning diet were significant, post hoc Bonferroni analysis was performed on all time points. The OGTT was analyzed with 2-factor repeated-measures ANOVA. Other parameters were analyzed using Student's *t* test (normally distributed data) or *t* test with Welch correction (normally distributed data with unequal variances). D'Agostino and Pearson omnibus normality tests were used to test for normality; data that were nonnormally distributed were log transformed and retested for normality. A Mann–Whitney *U* test on original data was applied when transformed data were also nonnormally distributed. The gene expression analyses were targeted and not corrected for

multiple comparisons (28, 29) and raw individual P values are used. Results are given as mean ± SEM, and P values <0.05 were considered significant.

Results

Male BC development

During the 3-wk postweaning period, BW, LM, and FM increased significantly in both GLU- and GLU + GAL–fed males (**Figure 1**A–C; see **Supplemental Table 3** for an overview of statistical parameters from 2-factor ANOVAs). Postweaning diet × time tended to affect BW ($P = 0.088$) and FM ($P = 0.096$), but postweaning diets had no significant effect overall ($P = 0.28$ for BW, $P = 0.28$ for FM). Similarly, LM was not affected significantly (Figure 1C; see Supplemental Table 3).

During the HFD period, BW, FM, and LM increased significantly in both GLU- and GLU + GAL–fed males (Figure 1A–C; see Supplemental Table 3). Postweaning diet × time tended to affect BW ($P = 0.094$), i.e., BW increased less in GLU + GAL–fed males than in those receiving GLU. There were, however, no significant postweaning diet × time interactions for FM or LM (Supplemental Table 3). Postweaning diets tended to affect BW during HFD feeding ($P = 0.061$), but postweaning diets had no effect on FM ($P = 0.19$). Postweaning diet affected LM significantly during the HFD period ($P = 0.007$); post hoc analysis indicated this was significant in weeks 8–12, but not subsequently (Figure 1C). Energy intake was higher among GLU + GAL–fed males than among GLU-fed males postweaning (Figure 1D), but the same during HFD-feeding (Figure 1E). Consequently, cumulative energy intakes were similar over the entire 12-wk study period (data not shown).

Female BC development

During the postweaning period, BW, FM, and LM of GLU- and GLU + GAL–fed females increased significantly (**Figure 2**A–C; see Supplemental Table 3). Postweaning diet × time was different for GLU- and GLU + GAL–fed females ($P = 0.031$). A trend for postweaning diet × time was seen in LM ($P = 0.058$), but not FM ($P = 0.62$). Overall, the diets had no significant effects on BW, LM, and FM. GLU + GAL–fed females consumed significantly more of the postweaning diet than did GLU-fed females (Figure 2D).

During the HFD period, BW, FM, and LM also increased significantly in both GLU- and GLU + GAL–fed females (Figure 2A–C; see Supplemental Table 3). Postweaning × time was significantly different during the HFD period for BW ($P < 0.0001$) and FM ($P < 0.0001$): BW and FM increased less in the GLU + GAL–fed females than in the GLU-fed females. Postweaning diets also had a significant effect on BW ($P = 0.008$) and FM ($P = 0.01$) during the HFD period: BW and FM were significantly lower in GLU + GAL–fed females from week 12 onwards. No postweaning × time effect was observed for LM ($P = 0.97$); LM gain was the same in both groups. However, although the postweaning diets had a significant impact on overall LM ($P = 0.021$), post hoc analysis showed this was nonsignificant at individual time points. Cumulatively, GLU + GAL–fed female mice consumed less HFD than did GLU-fed female mice (Figure 2E) and tended to have lower energy intakes over the entire 12-wk study (1.05 ± 0.02 Mcal/12 wk compared with 1.10 ± 0.02 Mcal/12 wk; $P = 0.09$).

Although patterns of development in BW, FM, and LM during the postweaning and HFD periods were similar for

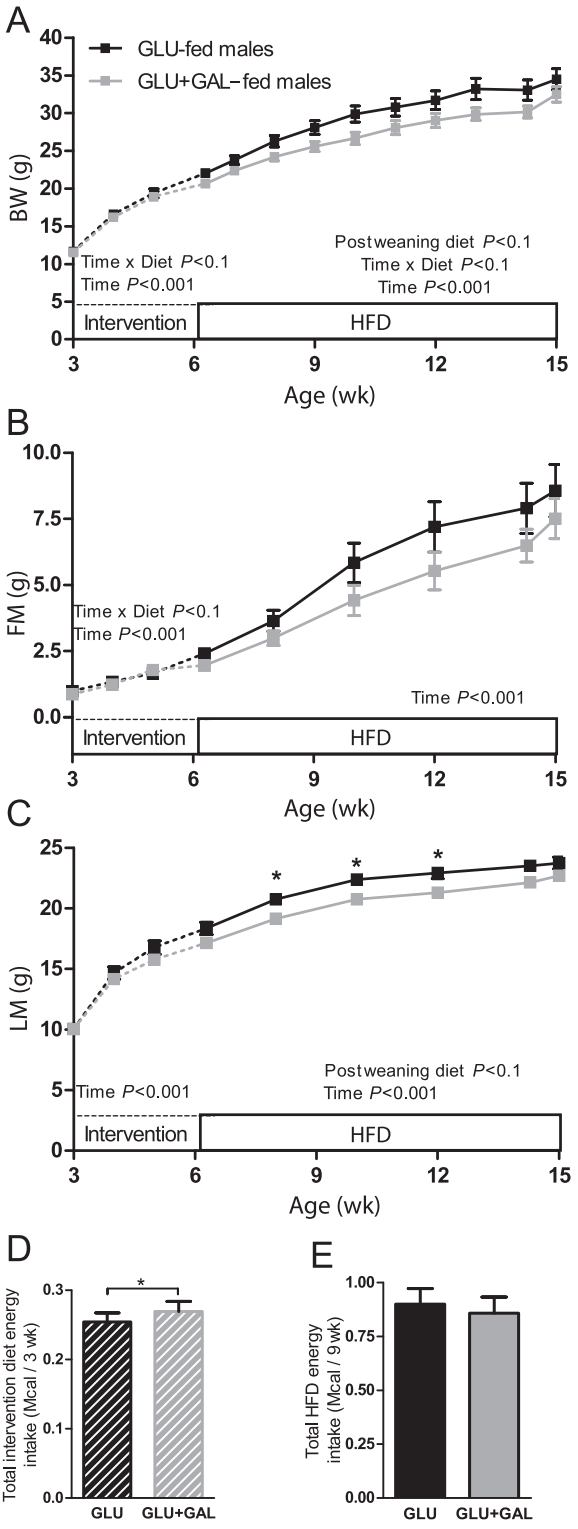

FIGURE 1 Longitudinal BW (A), FM (B), and LM (C) development and total energy intake during the postweaning (D) and HFD periods (E) for male mice fed GLU or GLU + GAL postweaning for 3 wk and thereafter an HFD for 9 wk. Group (postweaning GLU compared with GLU + GAL), time, and group-by-time effects were determined by repeated-measures ANOVA for BW, FM, and LM; postweaning and HFD periods were analyzed separately. Values are given as mean ± SEM, $n = 11$–12. ∗Groups differ, $P < 0.05$. Data on BW, FM, and energy intake from the control GLU-fed group have been published (22). BW, body weight; FM, fat mass; GLU, diet with 32 en% glucose; GLU + GAL, diet with 16 en% galactose and 16 en% glucose; HFD, high-fat diet; LM, lean mass.

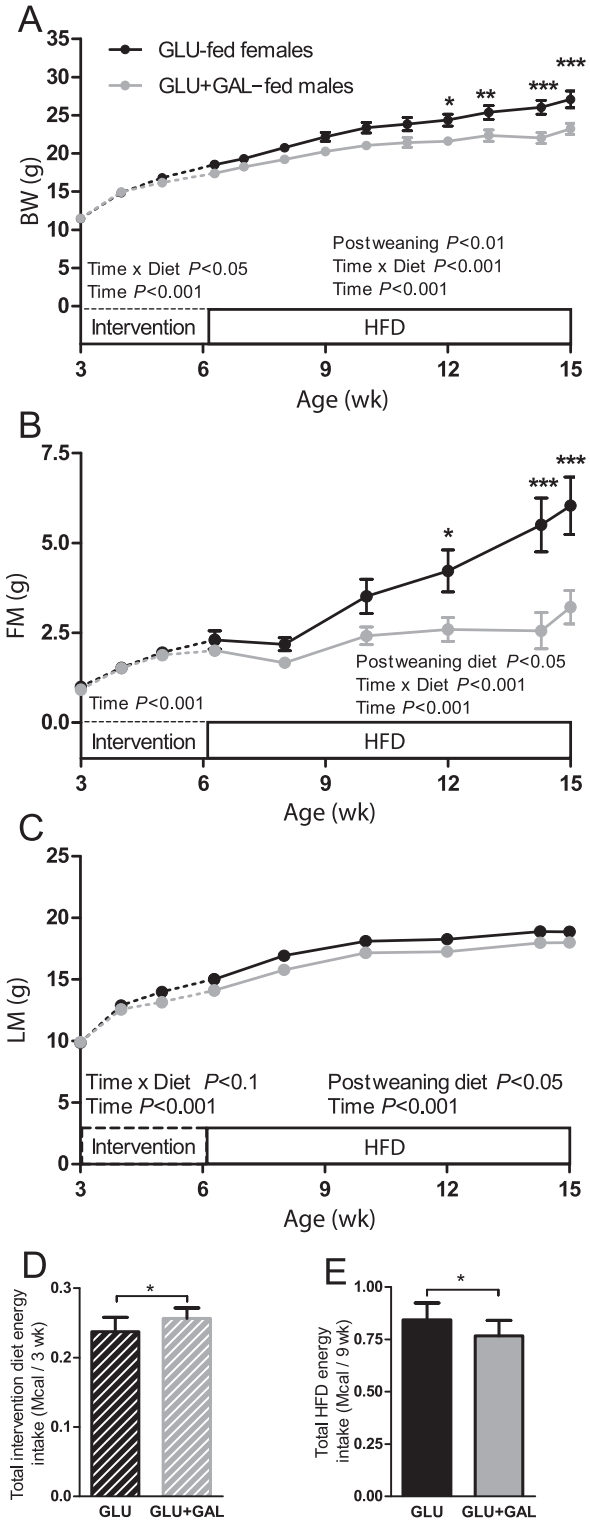

FIGURE 2 Longitudinal BW (A), FM (B), and LM (C) development and total energy intake during the postweaning (D) and HFD periods (E) for female mice fed GLU or GLU + GAL postweaning for 3 wk and thereafter an HFD for 9 wk. Group (postweaning GLU compared with GLU + GAL), time, and group-by-time effects were determined by repeated-measures ANOVA for BW, FM, and LM; postweaning and HFD periods were analyzed separately. Values are mean ± SEM, $n = 12$–14. *,**,***Groups differ: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Data on BW, FM, and energy intake from the control GLU-fed group have been published (22). BW, body weight; FM, fat mass; GLU, diet with 32 en% glucose; GLU + GAL, diet with 16 en% galactose and 16 en% glucose; HFD, high-fat diet; LM, lean mass.

both sexes, at the end of the study, diet affected BW and BC significantly only in females. Therefore, subsequent analyses focused only on females and, in particular, FM-related parameters.

Metabolic characterization of programming of females

GLU + GAL–fed females had less mesenteric WAT (**Figure 3**A) and gWAT (Figure 3B) than GLU-fed females. The mean serum leptin concentration in GLU + GAL–fed females was approximately one-third of the mean concentration in GLU-fed females (Figure 3C). Hepatic TG content was similar in both groups (Figure 3D), as was liver weight (Figure 3E). Blood glucose concentrations were also similar (Figure 3F), but serum insulin concentrations were lower in GLU + GAL–fed females (Figure 3G), indicating greater insulin sensitivity in GLU + GAL–fed females after the HFD period. Indeed, HOMA-IR, a surrogate marker for insulin resistance that is widely used but has its limitations (27), was also lower for GLU + GAL–fed females ($P = 0.03$; Figure 3H). Analysis of the incremental AUC from OGTTs performed 4 wk earlier (week 11) showed a trend for better glucose tolerance in GLU + GAL–fed females than in GLU-fed females ($P = 0.09$; **Figure 4**A, B). Immunohistological analysis of pancreata from GLU- and GLU + GAL–fed females (**Supplemental Figure 1**A, B) showed that both β-cell areas (Supplemental Figure 1C; $P = 0.37$) and masses (Figure 3I; $P = 0.12$) were, however, similar.

Because both energy intakes and serum leptin concentrations were different between GLU- and GLU + GAL–fed females (Figures 2D, E, and 3C), hypothalamic gene expression of orexigenic and anorexigenic transcripts and leptin signaling were examined. However, no significant differences were found between GLU- and GLU + GAL–fed females (**Figure 5**).

Histological and detailed molecular analyses of gWAT depots showed a trend for smaller unilocular adipocytes (diameter <50 μm) in GLU + GAL–fed females than in GLU-fed females ($P = 0.07$) (**Supplemental Figure 2**). Leptin gene expression (*Lep*) was lower in gWAT as well as sWAT of GLU + GAL–fed females (**Figures 6** and 7). Expression of mesoderm-specific transcript homolog protein (*Mest*), a marker for adipose expandability, was also lower among GLU + GAL–fed females in both WAT depots. However, no differences in gWAT expression of fatty acid binding protein 4 (*Fabp4*) and perilipin 1 (*Plin1*), adipocyte differentiation markers, were observed (Figure 6).

To explore differences associated with the lower HOMA-IR index, insulin signaling was also analyzed. Strikingly, insulin receptor substrate 2 (*Irs2*) expression was significantly lower in gWAT of GLU + GAL–fed females than in gWAT of GLU-fed females. Expression of insulin receptor (*Insr*) and *Irs1* in gWAT was the same (Figure 6). Insulin signaling–linked gene expression in sWAT was significantly affected: *Irs2* expression was significantly lower in GLU + GAL– than in GLU-fed females (Figure 7), and although *Insr* expression was similar in both groups, *Irs1* was lower in GLU + GAL–fed females (Figure 7). In gWAT, expression of genes associated with fatty acid synthesis and elongation, lipolysis and fatty acid oxidation, the tricarboxylic acid cycle, and carbohydrate metabolism was not affected by postweaning diet composition (Figure 6). Likewise, in sWAT, fatty acid oxidation and phosphoenolpyruvate carboxykinase 1 (*Pck1*) were not regulated differentially. Although hormone-sensitive lipase (*Lipe*) expression was significantly lower in GLU + GAL–fed females, other lipid metabolism–related genes [acetyl-CoA carboxylase 2 (*Acacb*), acyl-CoA

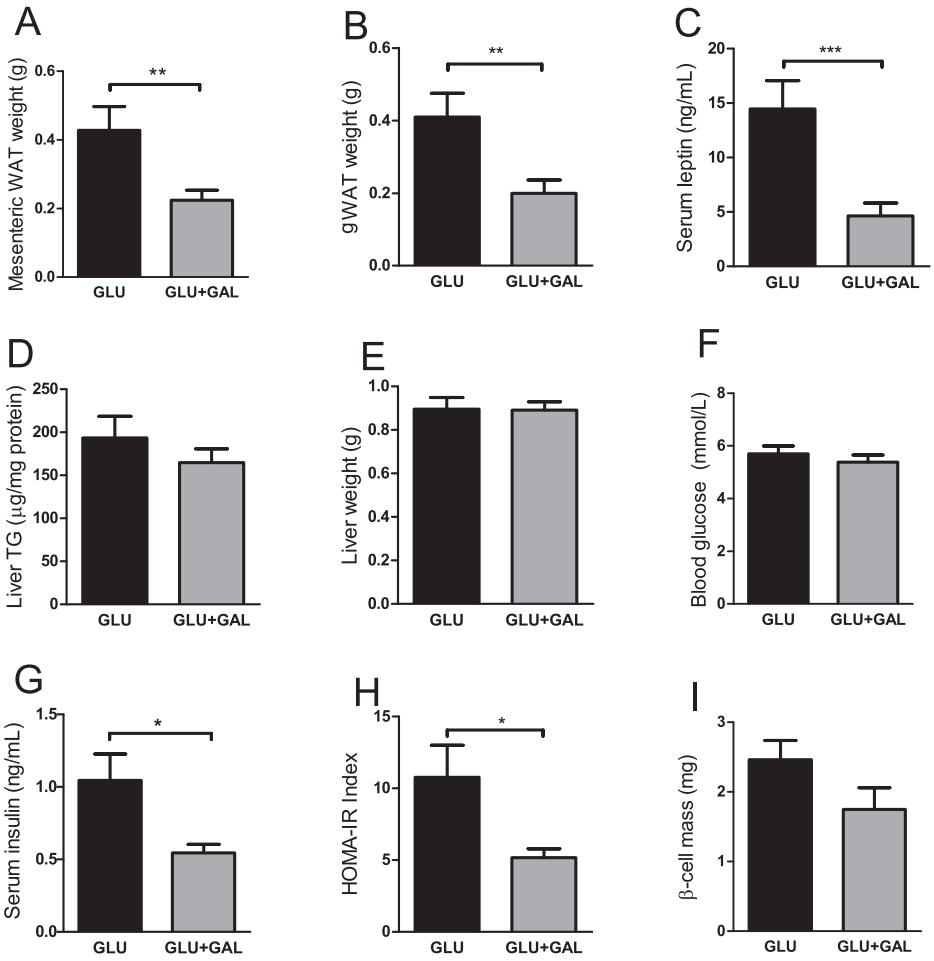

FIGURE 3 Mesenteric WAT (A), gWAT (B), serum leptin concentrations (C), hepatic TG content (D), liver weight (E), whole blood glucose concentrations (F), serum insulin concentrations (G), HOMA-IR (H), and pancreatic β-cell mass (I) in female mice fed GLU or GLU + GAL postweaning for 3 wk and thereafter a high-fat diet for 9 wk. Values are mean ± SEM, $n = 13$–14, $n = 6$ for β-cell mass. *,**,***Bracketed groups differ: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Data from the GLU-fed group have been published (22). GLU, diet with 32 en% glucose; GLU + GAL, diet with 16 en% galactose and 16 en% glucose; gWAT, gonadal white adipose tissue; WAT, white adipose tissue.

dehydrogenase (*Acadl*), and patatin like phospholipase domain containing 2 (*Pnpla2*)] were unaffected by the postweaning diet (Figure 7).

Discussion

In this study, we showed that replacing half of the glucose with galactose, compared with glucose alone, in an otherwise isocaloric 3-wk postweaning diet attenuated later-life HFD-induced adiposity in females. In males, only subtle or non-significant effects were seen; BW tended to be lower in male mice fed GLU + GAL postweaning than in male mice fed GLU ($P = 0.06$). Insulin resistance was lower in GLU + GAL–fed females in later life, with a likely role for insulin signaling via *Irs2* in WAT depots, based on reduced *Irs2* transcription levels. Such differences might be considered a form of nutritional programming, as defined by Lucas (30): "a stimulus or insult (a nutrient) operating at a critical or sensitive period of development results in a long-standing or life-long effect on the structure or function of the organism," even though the effects emerged partially during the postweaning period.

We explored brain and adipose tissues to find what might mediate galactose-related nutritional programming during the postweaning period in GLU + GAL–fed females. The fact that FI was higher during the postweaning period but lower during the HFD period for GLU + GAL–fed females than for GLU-fed females suggested the hypothalamus (Figure 5) could have a role, because it is the regulatory center for energy intake in the brain. Hypothalamic gene expression, related to satiety and to leptin signaling, has been shown to be susceptible to nutritional programming (31, 32). However, no changes in hypothalamic expression of the orexi- or anorexigenic (satiety-related) transcripts agouti-related peptide *Agrp*, neuropeptide Y *Npy*, and pro-opiomelanocortin *Pomc* were found. Also, there were no effects on expression of the leptin receptor (*Leprb*), or the leptin receptor activation marker suppressor of cytokine signaling 3 (*Socs3*), despite differences in circulating leptin concentrations between GLU- and GLU + GAL–fed females (Figure 3C). This contrasts with programming by dietary carbohydrates in nursing rats, where a high-carbohydrate formula during nursing was associated with upregulation of *Socs3* and *Leprb* in adult males (33). Also, exposure to a cafeteria diet during weaning affected directly energy balance–related gene expression in the hypothalami of male offspring (34). In female rats, hypothalamic programming has been reported during gestation (31, 32), but not in the postnatal period (33, 34). Although highly speculative, our results might

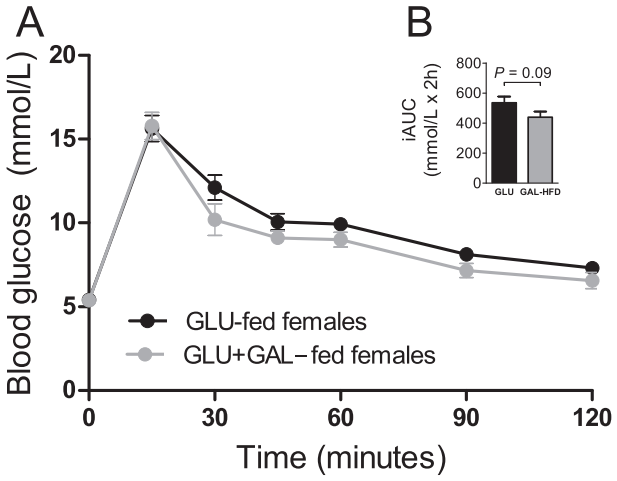

FIGURE 4 Blood glucose curves (A) and iAUC (B) from the oral-glucose-tolerance test performed in week 11 in female mice (fed GLU or GLU + GAL postweaning for 3 wk and thereafter an HFD for 5 wk). Values are mean ± SEM, n = 13–14. Data from the GLU-fed group have been published (22). GLU, diet with 32 en% glucose; GLU + GAL, diet with 16 en% galactose and 16 en% glucose; HFD, high-fat diet; iAUC, incremental AUC.

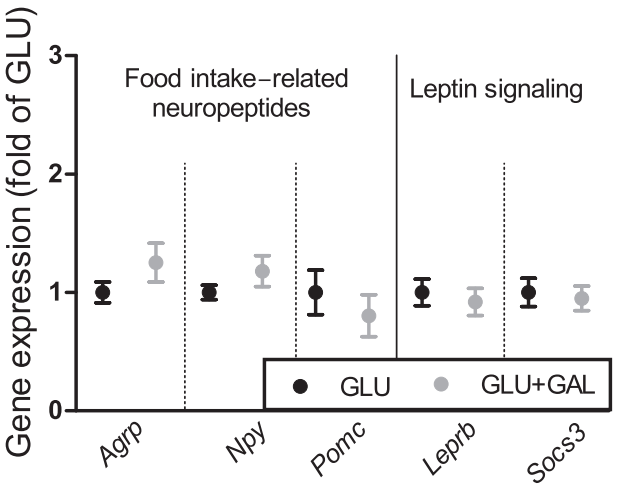

FIGURE 5 Gene expression in hypothalamus of female mice fed GLU or GLU + GAL postweaning for 3 wk and thereafter a high-fat diet for 9 wk. Values are mean ± SEM, n = 11–14. *Agrp*, agouti-related peptide; GLU, diet with 32 en% glucose; GLU + GAL, diet with 16 en% galactose and 16 en% glucose; *Leprb*, leptin receptor long isoform; *Npy*, neuropeptide Y; *Pomc*, pro-opiomelanocortin; *Socs3*, suppressor of cytokine signaling 3.

be explained if the postweaning period is not critical for hypothalamic programming in females.

Reduced adult adiposity observed in GLU + GAL–fed females might be due to differential effects on insulin signaling. The insulin response provoked by galactose (considering the direct effect) was small when compared with that of glucose [summarized in (35)]. Moreover, galactose has been reported to improve insulin sensitivity in rats (36). Thus, if also true in this nutritional model, insulin peaks in GLU + GAL–fed mice might be lower than in their GLU-fed counterparts. Differences in insulin release during a critical period of rapid growth and development might affect pancreatic development or the insulin response of peripheral tissues, ultimately affecting susceptibility to increased adiposity. Although we obtained no evidential support for the first notion, the second notion is supported by our findings in peripheral WAT, where *Irs2* was downregulated, suggesting less insulin was needed for adequate signal transduction.

Molecular analysis focused on females because proposed programming effects of GLU + GAL on adiposity were clear in this sex. A sex-dependent difference in metabolic programming has been seen previously (37–41). This may be due to (1 of)

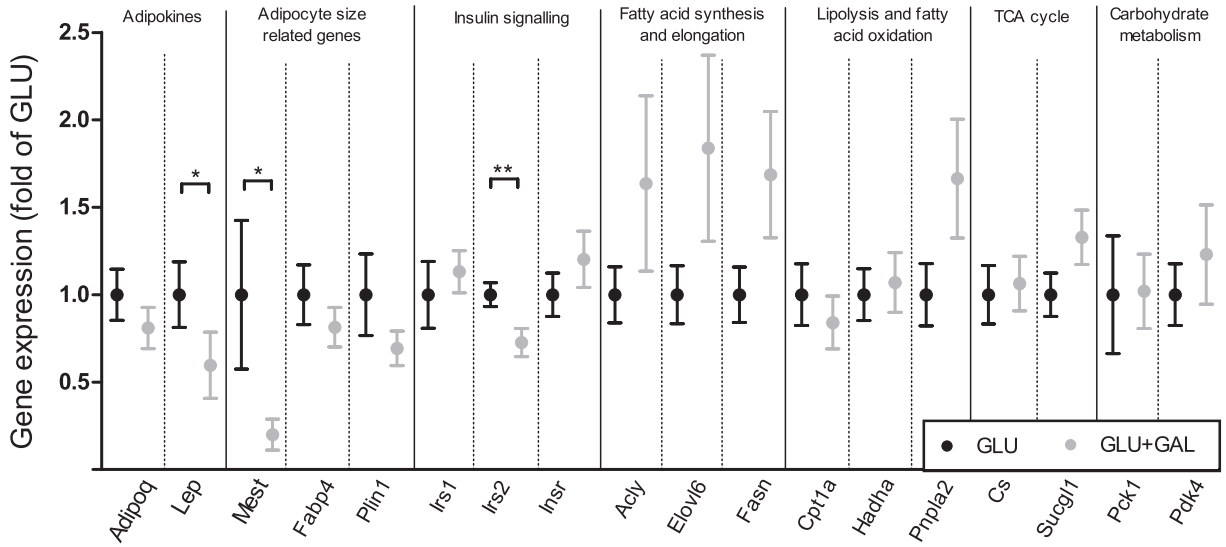

FIGURE 6 Gene expression in gonadal white adipose tissue of female mice fed GLU or GLU + GAL postweaning for 3 wk and thereafter a high-fat diet for 9 wk. *,**Bracketed gene differs between groups: *$P < 0.05$, **$P < 0.01$. Values are mean ± SEM, n = 9–13. *Acly*, ATP-citrate lyase; *Adipoq*, adiponectin; *Cpt1a*, carnitine palmitoyltransferase I; *Cs*, citrate synthase; *Elovl6*, elongation of long-chain fatty acids family member 6; *Fabp4*, fatty acid binding protein 4; *Fasn*, fatty acid synthase; GLU, diet with 32 en% glucose; *Hadha*, hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase; *Insr*, insulin receptor; *Irs1/2*, insulin receptor substrate 1/2; *Lep*, leptin; *Mest*, mesoderm-specific transcript homolog protein; *Pck1*, phosphoenolpyruvate carboxykinase 1; *Pdk4*, pyruvate dehydrogenase kinase 4; *Plin1*, perilipin 1; *Pnpla2*, patatin like phospholipase domain containing 2; *Sucgl1*, succinate CoA ligase subunit α; TCA, tricarboxylic acid.

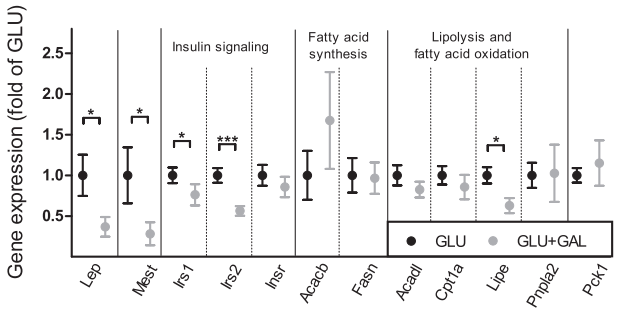

FIGURE 7 Gene expression in subcutaneous white adipose tissue of female mice fed GLU or GLU + GAL postweaning for 3 wk and thereafter a high-fat diet for 9 wk.*,***Bracketed gene differs between groups: $^{*}P < 0.05$, $^{***}P < 0.001$. Values are mean ± SEM, $n = 10$–14. *Acacb*, acetyl-CoA carboxylase 2; *Acadl*, acyl-CoA dehydrogenase; *Cpt1a*, carnitine palmitoyltransferase I; *Fasn*, fatty acid synthase; GLU, diet with 32 en% glucose; GLU + GAL, diet with 16 en% galactose and 16 en% glucose; *Insr*, insulin receptor; *Irs1/2*, insulin receptor substrate 1/2; *Lep*, leptin; *Lipe*, hormone-sensitive lipase; *Mest*, mesoderm-specific transcript homolog protein; *Pck1*, phosphoenolpyruvate carboxykinase 1; *Pnpla2*, patatin like phospholipase domain containing 2.

the various differences that exist between males and females, including circulating steroid hormones and BC (42) and growth patterns. Independent of the origins of such differences, our results highlight the fact that sex-related dimorphism should be considered when studying metabolic programming effects in any species, including humans.

Our results suggest that a mixture of glucose and galactose, mimicking lactose, given in the postnursing period, might protect against HFD-induced insulin resistance in a mouse model for human nutrition. It remains to be seen whether this finding can be translated to humans and whether lactose, indeed, explains the beneficial health effects of extended breastfeeding during the transition to solid foods. Some support for this theory has been provided by a meta-analysis of prospective cohort studies showing that consumption of dairy products, the major source of dietary lactose, reduces the risk of being overweight or obese in both children and adolescents (43). Another meta-analysis found an inverse relation between dairy consumption and adiposity in adolescents, although no effect was found in pre- and school-aged children (44). Although it is tempting to speculate that these effects are due to lactose, other dairy components such as calcium and high-quality protein might also have a role (43), although adding lactose to an HFD was associated with reduced weight gain and adiposity in rats, again favoring the direct effect of galactose (45).

We used glucose and galactose in a 1:1 ratio rather than lactose to ensure lactase deficiency did not interfere with our results and because galactose metabolism is influenced by co-ingestion with glucose. Plasma galactose concentrations are lower when consumed with equimolar amounts of glucose (46) because of increased splanchnic clearance (47). This is important in potential future applications, especially where galactose and glucose are used in preference to lactose, because the majority of adult humans (~70%) are lactase deficient (48).

In conclusion, this study showed that replacing glucose with galactose in a postweaning diet, in a 1:1 ratio (mimicking lactose), had beneficial metabolic programming effects in female mice, over glucose alone, characterized by lower adiposity, BW, HOMA-IR index, and expression of insulin signaling components in WAT. Future research is needed to elucidate the mechanisms underlying these benefits and the impact of such dietary modification on adiposity, insulin sensitivity, and energy balance in adulthood, as well as its translatability to humans. Nevertheless, this knowledge could be useful for determining (ga)lactose concentrations in infant and toddler formulas. Similarly, consumption of lactose—in those who are lactose tolerant as adults—might be beneficial over glucose alone, owing to the persistence of galactose in the diet.

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References

1. Barker DJ. The fetal and infant origins of adult disease. BMJ 1990;301:1111.
2. Painter RC, Roseboom TJ, Bleker OP. Prenatal exposure to the Dutch famine and disease in later life: an overview. Reprod Toxicol 2005;20:345–52.
3. Koletzko B, Brands B, Grote V, Kirchberg FF, Prell C, Rzehak P, Uhl O, Weber M; Early Nutrition Programming Project. Long-term health impact of early nutrition: the power of programming. Ann Nutr Metab 2017;70:161–9.
4. Baars A, Oosting A, Engels E, Kegler D, Kodde A, Schipper L, Verkade HJ, van der Beek EM. Milk fat globule membrane coating of large lipid droplets in the diet of young mice prevents body fat accumulation in adulthood. Br J Nutr 2016;115:1930–7.
5. Oosting A, Kegler D, Wopereis HJ, Teller IC, van de Heijning BJ, Verkade HJ, van der Beek EM. Size and phospholipid coating of lipid droplets in the diet of young mice modify body fat accumulation in adulthood. Pediatr Res 2012;72:362–9.
6. Oosting A, van Vlies N, Kegler D, Schipper L, Abrahamse-Berkeveld M, Ringler S, Verkade HJ, van der Beek EM. Effect of dietary lipid structure in early postnatal life on mouse adipose tissue development and function in adulthood. Br J Nutr 2014;111:215–26.
7. Wielinga PY, Harthoorn LF, Verschuren L, Schoemaker MH, Jouni ZE, van Tol EA, Kleemann R, Kooistra T. Arachidonic acid/docosahexaenoic acid-supplemented diet in early life reduces body weight gain, plasma lipids, and adiposity in later life in ApoE*3Leiden mice. Mol Nutr Food Res 2012;56:1081–9.
8. Shaoul R, Tiosano D, Hochberg Z. Evo-devo of child growth: the role of weaning in the transition from infancy to childhood. Crit Rev Food Sci Nutr 2016;56:887–95.
9. Stephen A, Alles M, de Graaf C, Fleith M, Hadjilucas E, Isaacs E, Maffeis C, Zeinstra G, Matthys C, Gil A. The role and requirements of digestible dietary carbohydrates in infants and toddlers. Eur J Clin Nutr 2012;66:765–79.
10. Harrison M, Brodribb W, Hepworth J. A qualitative systematic review of maternal infant feeding practices in transitioning from milk feeds to family foods. Matern Child Nutr 2017;13:e12360.
11. Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. Pediatr Clin North Am 2013;60:49–74.
12. Ocké MC, van Rossum CTM, Fransen HP, Buurma-Rethans EJM, de Boer EJ, Brants HAM, Niekerk EM, van der Laan JD, Drijvers JJMM, Ghameshlou Z. Dutch National Food Consumption Survey – young children 2005/2006. RIVM-Report 350070001. Bilthoven: National Institute for Public Health and the Environment (RIVM); 2008.
13. van Rossum CTM, Fransen HP, Verkaik-Kloosterman J, Buurma-Rethans EJM, Ocké MC. Dutch National Food Consumption Survey 2007–2010. Diet of children and adults 7 to 69 years. RIVM-Report

350050006. Bilthoven: National Institute for Public Health and the Environment (RIVM); 2011.
14. Sluik D, Engelen AI, Feskens EJ. Fructose consumption in the Netherlands: the Dutch National Food Consumption Survey 2007–2010. Eur J Clin Nutr 2015;69:475–81.
15. Fewtrell M, Bronsky J, Campoy C, Domellof M, Embleton N, Fidler Mis N, Hojsak I, Hulst JM, Indrio F, Lapillonne A, et al. Complementary feeding: a position paper by the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Committee on Nutrition. J Pediatr Gastroenterol Nutr 2017;64:119–32.
16. Owen CG, Martin RM, Whincup PH, Smith GD, Cook DG. Effect of infant feeding on the risk of obesity across the life course: a quantitative review of published evidence. Pediatrics 2005;115:1367–77.
17. von Kries R, Koletzko B, Sauerwald T, von Mutius E, Barnert D, Grunert V, von Voss H. Breast feeding and obesity: cross sectional study. BMJ 1999;319:147–50.
18. Yan J, Liu L, Zhu Y, Huang GW, Wang PP. The association between breastfeeding and childhood obesity: a meta-analysis. BMC Public Health 2014;14:1267.
19. Srinivasan M, Mitrani P, Sadhanandan G, Dodds C, Shbeir-ElDika S, Thamotharan S, Ghanim H, Dandona P, Devaskar SU, Patel MS. A high-carbohydrate diet in the immediate postnatal life of rats induces adaptations predisposing to adult-onset obesity. J Endocrinol 2008;197:565–74.
20. D'Alessandro ME, Oliva ME, Ferreira MR, Selenscig D, Lombardo YB, Chicco A. Sucrose-rich feeding during rat pregnancy-lactation and/or after weaning alters glucose and lipid metabolism in adult offspring. Clin Exp Pharmacol Physiol 2012;39:623–9.
21. Regnault TRH, Gentili S, Sarr O, Toop CR, Sloboda DM. Fructose, pregnancy and later life impacts. Clin Exp Pharmacol Physiol 2013;40:824–37.
22. Bouwman LMS, Fernández-Calleja JMS, Swarts HJM, van der Stelt I, Oosting A, Keijer J, van Schothorst EM. No adverse programming by post-weaning dietary fructose of body weight, adiposity, glucose tolerance, or metabolic flexibility. Mol Nutr Food Res 2018;62:1700315.
23. Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76a rodent diet. J Nutr 1993;123:1939–51.
24. Hoevenaars FP, van Schothorst EM, Horakova O, Voigt A, Rossmeisl M, Pico C, Caimari A, Kopecky J, Klaus S, Keijer J. BIOCLAIMS standard diet (BIOsd): a reference diet for nutritional physiology. Genes Nutr 2012;7:399–404.
25. Voigt A, Agnew K, van Schothorst EM, Keijer J, Klaus S. Short-term, high fat feeding-induced changes in white adipose tissue gene expression are highly predictive for long-term changes. Mol Nutr Food Res 2013;57:1423–34.
26. Tinsley FC, Taicher GZ, Heiman ML. Evaluation of a quantitative magnetic resonance method for mouse whole body composition analysis. Obes Res 2004;12:150–60.
27. van Dijk TH, Laskewitz AJ, Grefhorst A, Boer TS, Bloks VW, Kuipers F, Groen AK, Reijngoud DJ. A novel approach to monitor glucose metabolism using stable isotopically labelled glucose in longitudinal studies in mice. Lab Anim 2013;47:79–88.
28. Rothman KJ. No adjustments are needed for multiple comparisons. Epidemiology 1990;1:43–6.
29. Saville DJ. Multiple comparison procedures: the practical solution. Am Stat 1990;44:174–80.
30. Lucas A. Programming not metabolic imprinting. Am J Clin Nutr 2000;71:602.
31. Garcia AP, Palou M, Priego T, Sanchez J, Palou A, Pico C. Moderate caloric restriction during gestation results in lower arcuate nucleus NPY- and αMSH-neurons and impairs hypothalamic response to fed/fasting conditions in weaned rats. Diabetes Obes Metab 2010;12:403–13.
32. Ikenasio-Thorpe BA, Breier BH, Vickers MH, Fraser M. Prenatal influences on susceptibility to diet-induced obesity are mediated by altered neuroendocrine gene expression. J Endocrinol 2007;193:31–7.
33. Srinivasan M, Mahmood S, Patel MS. Metabolic programming effects initiated in the suckling period predisposing for adult-onset obesity cannot be reversed by calorie restriction. Am J Physiol Endocrinol Metab 2013;304:E486–E94.
34. Castro H, Pomar CA, Pico C, Sanchez J, Palou A. Cafeteria diet overfeeding in young male rats impairs the adaptive response to fed/fasted conditions and increases adiposity independent of body weight. Int J Obes 2015;39:430–7.
35. Charrière N, Loonam C, Montani JP, Dulloo AG, Grasser EK. Cardiovascular responses to sugary drinks in humans: galactose presents milder cardiac effects than glucose or fructose. Eur J Nutr 2017;56:2105–13.
36. Stahel P, Kim JJ, Xiao C, Cant JP. Of the milk sugars, galactose, but not prebiotic galacto-oligosaccharide, improves insulin sensitivity in male Sprague-Dawley rats. PLoS One 2017;12:e0172260.
37. Choi GY, Tosh DN, Garg A, Mansano R, Ross MG, Desai M. Gender-specific programmed hepatic lipid dysregulation in intrauterine growth-restricted offspring. Am J Obstet Gynecol 2007;196:477. e1–7.
38. Dearden L, Balthasar N. Sexual dimorphism in offspring glucose-sensitive hypothalamic gene expression and physiological responses to maternal high-fat diet feeding. Endocrinology 2014;155:2144–54.
39. Samuelsson AM, Matthews PA, Jansen E, Taylor PD, Poston L. Sucrose feeding in mouse pregnancy leads to hypertension, and sex-linked obesity and insulin resistance in female offspring. Front Physiol 2013;4:14.
40. Sun B, Purcell RH, Terrillion CE, Yan J, Moran TH, Tamashiro KL. Maternal high-fat diet during gestation or suckling differentially affects offspring leptin sensitivity and obesity. Diabetes 2012;61:2833–41.
41. van Straten EM, Bloks VW, van Dijk TH, Baller JF, Huijkman NC, Kuipers I, Verkade HJ, Plosch T. Sex-dependent programming of glucose and fatty acid metabolism in mouse offspring by maternal protein restriction. Gend Med 2012;9:166–79. e13.
42. Wells JC. Sexual dimorphism of body composition. Best Pract Res Clin Endocrinol Metab 2007;21:415–30.
43. Lu L, Xun P, Wan Y, He K, Cai W. Long-term association between dairy consumption and risk of childhood obesity: a systematic review and meta-analysis of prospective cohort studies. Eur J Clin Nutr 2016;70:414–23.
44. Dror DK. Dairy consumption and pre-school, school-age and adolescent obesity in developed countries: a systematic review and meta-analysis. Obes Rev 2014;15:516–27.
45. Goseki-Sone M, Maruyama R, Sogabe N, Hosoi T. Effects of dietary lactose on long-term high-fat-diet-induced obesity in rats. Obesity 2007;15:2605–13.
46. Williams CA, Phillips T, Macdonald I. The influence of glucose on serum galactose levels in man. Metabolism 1983;32:250–6.
47. Sunehag AL, Haymond MW. Splanchnic galactose extraction is regulated by coingestion of glucose in humans. Metabolism 2002;51:827–32.
48. Heyman MB. Lactose intolerance in infants, children, and adolescents. Pediatrics 2006;118:1279–86.