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

Lianne MS Bouwman, José MS Fernández-Calleja, Inge van der Stelt, Annemarie Oosting, Jaap Keijer, and Evert M van Schothorst

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.


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
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.

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/6J RccHsd mice were given a starch-based diet containing additional monosaccharides at 32 energy percent (en%) from glucose, or 16 en% galactose + 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 mix 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. Nes 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 ± 1°C; 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 fixated in paraformaldehyde. Hypothalami were based on density in PBS; both organswer were weighed and (gWAT) was excised; left fat pads were snap-frozen in liquid nitrogen, whereas right fat pads were weighed and fixed 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 (ChrysalChem) following the manufacturer's instructions. Serum measurements were done in duplicate. HOMA-IR was calculated from serum insulin (in milligrams 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/soyamyl-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-Blst (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). Primer sequences and annealing temperatures are given in Supplemental Table 2. cDNA was normalized against housekeeping genes, and Rps15 for sWAT; and B2m and Rps15 for hypothalamus. 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 1A–C; see Supplemental Table 3 for an overview of statistical parameters from 2-factor ANOVAs). Postweaning diet \( \times \) 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). Postweaning diet \( \times \) time tended to affect BW (\( P = 0.088 \)), i.e., BW increased less in GLU + GAL–fed males than in those receiving GLU. There were, however, no significant postweaning diet \( \times \) 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 2A–C; see Supplemental Table 3). Postweaning diet \( \times \) time was different for GLU- and GLU + GAL–fed females (\( P = 0.031 \)). A trend for postweaning diet \( \times \) 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). Postweaning \( \times \) 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 \( \times \) 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.](image-url)
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 3A) 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 4A, B). Immunohistological analysis of pancreata from GLU- and GLU + GAL–fed females (Supplemental Figure 1A, 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 (Insr2) expression was significantly lower in gWAT of GLU + GAL–fed females than in gWAT of GLU-fed females. Expression of insulin receptor (Insr1) and Insr1 in gWAT was the same (Figure 6). Insulin signaling–linked gene expression in sWAT was significantly affected: Insr2 expression was significantly lower in GLU + GAL– than in GLU-fed females (Figure 7), and although Insr1 expression was similar in both groups, Insr1 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
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.

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)
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.

Acknowledgments

We acknowledge Hans Swarts, Irini Saridakis, Quinten Ducarnon, and Ardy Lizuardi for their help in laboratory analyses. The authors’ responsibilities were as follows—LMSB, JMSFC, AO, JK, and EMvS: designed the research; LMSB, JMSFC, and Ivds: performed the experiments; LMSB and EMvS: analyzed the data; LMSB, EMvS, AO, and JK: wrote the draft and final manuscript; LMSB: had primary responsibility for the final content; and all authors: read and approved the final manuscript.

References

1148 Bouwman et al.


47. Sunehag AL, Haymond MW. Splanchnic galactose extraction is regulated by congestion of glucose in humans. Metabolism 2002;51:827–32.