Vitamin A Supplementation during Suckling and Postweaning Periods Attenuates the Adverse Metabolic Effects of Maternal High-Fat Diet Consumption in Sprague-Dawley Rats

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ABSTRACT

Background: Vitamin A (VA) has been demonstrated to be a regulator of adipose tissue (AT) development in adult obese models. However, little is known about the effect of VA on obesity-associated developmental and metabolic conditions in early life.

Objectives: We aimed to assess the effects of dietary VA supplementation during suckling and postweaning periods on the adiposity and metabolic health of neonatal and weanling rats from mothers consuming a high-fat diet (HFD).

Methods: Pregnant Sprague-Dawley rats were fed a normal-fat diet (NFD; 25% fat; $n = 2$) or an HFD (50% fat; $n = 2$), both with 2.6 mg VA/kg. Upon delivery, half of the rat mothers were switched to diets with supplemented VA at 129 mg/kg, whereas the other half remained at 2.6 mg VA/kg. Four groups of rat pups were designated as NFD, NFD + VA, HFD, and HFD + VA, respectively. At postnatal day (P)14, P25, and P35, pups ($n = 4$ or 3/group) were killed. Body weight (BW), visceral white AT (WAT) mass, brown AT (BAT) mass, uncoupling protein 1 mRNA expression in BAT, serum glucose, lipids, adipokines, and inflammatory biomarkers, as well as serum and AT redox status were assessed.

Results: Rat pups in the HFD group exhibited significantly higher BW, WAT mass, and serum glucose and leptin but reduced BAT mass compared with the NFD group. Without affecting the dietary intake, supplementing the HFD with VA significantly reduced the BW and WAT mass of pups but increased the BAT mass, significantly lowered the systemic and WAT oxidative stress, and modulated serum adipokines and lipids to some extent.

Conclusions: VA supplementation during suckling and postweaning periods attenuated metabolic perturbations caused by excessive fat intake. Supplementing maternal or infant obesogenic diets with VA or establishing a higher RDA of VA for specific populations should be studied further for managing overweight/obesity in early life. *Curr Dev Nutr* 2020;4:nzaa111.

Introduction

According to the CDC, since the 1970s, the number of children and adolescents affected by obesity in the United States has tripled, with the prevalence of obesity among 2- to 5-y-olds at ∼14% (1). A joint analysis done by UNICEF, the WHO, and the World Bank indicated that the number of overweight children <5 y old is expected to reach a prevalence of 10% by the year 2025 (2). The upward trend in the number of overweight children is seen across high-income, middle-income, and low-income countries. Childhood obesity has also been reported to be closely related to maternal obesity. It was estimated that 30% of pregnant women in the United States are obese (3) and ∼40% of women gain an excessive amount of weight during pregnancy (4), for both of

which an unhealthy dietary pattern (5, 6), such as a high-fat diet (HFD) (7), may be a significant contributor. Both maternal obesity and excessive gestational weight gain may program obesity and/or other chronic diseases in offspring during infancy, childhood, and later in life (8). As such, research exploring effects of dietary or nutritional factors during gestation and/or lactation on adipose tissue (AT) development and associated metabolic conditions in the early life stage is of great importance.

Previous studies have well established that, in adults, the essential micronutrient vitamin A (VA) is a key regulator of lipid metabolism and AT development (9–11). In adult obese rats, chronic dietary VA supplementation was found to reduce body weight (BW) gain, adiposity, and AT mass (12, 13). VA supplementation was reported to increase fat mobilization from the white adipose tissue (WAT) while decreasing its

accumulation (14). Using adult rodent models, it was shown that retinoic acid (RA), the active metabolite of VA, inhibited adipogenesis (14, 15) while stimulating angiogenesis and apoptosis (16) of WAT. In addition, RA increased the adaptive thermogenesis of brown adipose tissue (BAT) via regulating the expression of uncoupling proteins (17) and induced the browning of WAT (18) or remodeling of WAT to BAT (19). Moreover, both dietary VA supplementation and acute RA treatment were found to modulate the concentrations of leptin and adiponectin in obese adult rats (20–23). Interestingly, it was reported that the "machinery" required for the action of VA, including retinoic acid receptors, retinoid X receptors, and enzymes involved in VA metabolism, is all expressed in the AT (24), informing the potentially critical role of VA in AT metabolism.

Despite those remarkable findings in adult models, the effect of VA on obesity-associated developmental and metabolic conditions in early life has scarcely been studied. In a recent study by Wang et al. (25), it was shown that maternal VA supplementation during pregnancy and lactation reduced the BW, WAT and BAT mass, and size of adipocytes in C57BL/6 mice offspring challenged with an HFD from 1 mo to 5 mo old. However, maternal obesity was not induced or considered in that study. Given the high prevalence of obesity or excessive weight gain in pregnant and lactating women, it is important to understand how VA may interact with maternal obesogenic diets.

Therefore, the objective of this exploratory study was to assess, in neonatal and weanling rat offspring from mothers consuming an HFD, how VA supplementation will modulate their BW, adiposity, and obesity-associated metabolic conditions, including redox and inflammatory status, glucose, lipid profile, and adipokines. VA supplementation through enriching the maternal diet was chosen as the supplementation route for neonates, because it was shown to have a significantly greater sustained effect on improving neonatal VA status than VA treatment directly given to neonates (26). Considering its potential teratogenic effect, maternal VA supplementation was administered during lactation, but not the gestational period. It was hypothesized that VA supplementation would significantly reduce the adiposity and improve the metabolic conditions of neonatal and weanling rats receiving excessive fat intake.

Methods

Animal experiment

Animal protocols were approved by the Institutional Animal Use and Care Committee of the University of Alabama and followed throughout the study. Pregnant Sprague-Dawley rats were purchased from Charles River Laboratories and arrived on their second day of gestation. Rats were housed individually with a 12-h light/dark cycle with free access to food and water. After a 3-d acclimation period, rats were randomly assigned to either a normal-fat diet (NFD; 25% kcal from fat; $n = 2$) or an HFD (50% kcal from fat; $n = 2$), both with 2.6 mg/kg of VA as retinyl acetate, which is adequate for the health maintenance of pregnant mothers and for fetal development. Upon delivery, pups were transferred among litters in the same cohort to achieve the same number per litter; half of the rat mothers were switched to diets with supplemented VA at 129 mg/kg (designated as the NFD + VA and the HFD + VA group, respectively), whereas the other half remained at 2.6 mg VA/kg

(designated as the NFD and the HFD group, respectively). All the experimental diets were purchased from Research Diets, Inc.

At postnatal day (P)14 and P25, 4 pups/group were weighed and killed with carbon dioxide. Blood was collected from the vena cava. Visceral WAT, BAT, and liver were excised, weighed, and rapidly frozen in liquid nitrogen. Serum was obtained after centrifugation of blood samples at $1500 \times g$ for 20 min at $4°C$ and stored at $−80°C$; tissue samples were stored at $−80°C$ until analysis. The remaining weanling pups ($n = 3$/group) started to receive the same diets as their respective mothers until they were killed at P35 with blood, liver, visceral WAT, and BAT harvested. In addition, at P14, stomach was also collected to obtain milk samples. Throughout the study, diets were administered once a day with daily food consumption recorded.

Serum and tissue analysis
Redox status.

Oxidative stress. According to the TBAR assay as previously described (27), malondialdehyde (MDA), a product of lipid peroxidation and a biomarker of oxidative stress in serum, WAT, and BAT samples, was quantified. Results are expressed as mM MDA.

Antioxidant capacity. Serum, WAT, and BAT were deproteinated according to a published method using methanol:acetonitrile:acetone (1:1:1, by vol) added to samples in a ratio of 1:4 (vol:vol) (28). The oxygen radical absorbance capacity assay on a FLUOstar Optima plate reader (BMG Labtech) was applied to measure the antioxidant capacity (AC) (29). The compound 2,2-azobis(2-amidinopropane) dihydrochloride was used as the peroxyl radical generator and Trolox, a water-soluble analog of vitamin E, served as the reference antioxidant standard. Results are expressed as μM Trolox equivalents.

Serum glucose, lipids, adipokines, and inflammatory biomarkers.
Serum samples from P25 were assessed for glucose, lipids, leptin, adiponectin, and inflammatory biomarkers. Glucose concentration was measured using glucose oxidase on a Stanbio Sirus analyzer. Concentrations of total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol were measured directly using a Stanbio Sirus analyzer. Adiponectin concentration was assessed using a Millipore Rat Adiponectin ELISA and leptin was measured using a Millipore Rat Leptin ELISA. Concentrations of inflammatory biomarkers including IL-10, IL-6, keratinocyte chemoattractant/growth-regulated oncogene (KC/GRO; also known as neutrophil-activating protein 3 or NAP-3), and TNF-α were measured using a MesoScale Discovery Rat Proinflammatory Panel 2 kit.

Uncoupling protein 1 mRNA expression in BAT.
For uncoupling protein 1 (UCP-1) mRNA determination in rat pups' BAT, samples from P25 and P35 killing were used, but not those from P14 killing owing to inadequate tissue amounts for analysis. Total RNA was extracted from BAT samples using Trizol (Invitrogen) and cDNA was prepared by using a cDNA synthesis kit (QuantaBio). The equivalent of 1 μg RNA, as cDNA, was used for real-time qPCR analysis. The primer designed to detect UCP-1 mRNA expression was rat UCP-1 (NM_012682.2), 5'-AGAAGGATTGCCGAAACTGTAC-3' (forward) and 5'-AGATCTTGCTTCCCAAAGAGG-3' (reverse). β-

Actin was used as the housekeeping gene. The $2^{-\Delta\Delta CT}$ method (30) was used to compare the relative UCP-1 mRNA expression among groups.

VA concentration in serum, milk, WAT, and BAT.

The total retinol concentrations in pups' serum, WAT, and BAT collected at all 3 time points as well as in milk samples separated from pups' stomach harvested at P14 were measured by ultra-performance liquid chromatography (UPLC) (Acquity UPLC System; Waters). These data were published in an article that addressed a different research question (31). Part of these data will be cited in the Results section as supporting data.

Statistical analysis

Data are reported as means ± SEMs. Differences between groups, *P* value < 0.05, were determined by using 1-factor ANOVA followed by Bonferroni post test in Prism software (GraphPad). A Pearson correlation test in GraphPad Prism was applied to analyze the correlation among measures.

Results

Dietary intakes

During pregnancy, the mean daily food intake of rat mothers consuming the HFD was significantly lower than that of mothers on the NFD (17.19 ± 1.63 g/d compared with 24.17 ± 2.87 g/d; *P* < 0.01), but the daily fat intake was significantly higher (4.64 ± 0.44 g/d compared with 2.90 ± 0.34 g/d; *P* < 0.01). The differing dietary intakes did not result in significant differences in the birth weight of rat pups in the 2 cohorts (6.76 ± 0.99 g in the HFD cohort compared with 7.56 ± 0.88 g in the NFD cohort).

During lactation, no significant difference in dietary intake was noted between VA-supplemented and nonsupplemented dams. All groups had a daily food intake increasing from ∼25 g/d on lactation day 1 to ∼80 g/d on lactation day 22. Rat pups remaining after P25 were weaned and received the same diet that their respective mother consumed. During the postweaning stage (P25–P35), no significant difference in the mean daily food intake was noted (11.04 ± 0.70 g/d, 12.87 ± 0.18 g/d, 11.64 ± 1.94 g/d, and 12.45 ± 1.62 g/d for pups in NFD, NFD + VA, HFD, and HFD + VA, respectively).

Milk and serum VA concentration

As presented in our previous publication (31), the total retinol concentration in the milk separated from rat pups' stomach was significantly higher in the NFD + VA and HFD + VA groups than in their respective controls (∼20-fold increase; *P* < 0.05), indicating that rat pups in VA-supplemented groups received significantly more VA. No significant difference was noted between the NFD and the HFD group or between the NFD + VA and the HFD + VA group. The serum total retinol concentration was also significantly (*P* < 0.01) higher in the VA-supplemented groups than in the nonsupplemented groups at all 3 time points, confirming the effectiveness of VA supplementation in raising pups' VA status.

BW and adiposity

At P14 and P25, the BW and visceral WAT mass of pups in the HFD group were significantly (*P* < 0.01) higher than those in the NFD group (**Figure 1**A, B). Comparatively, those measures were significantly decreased in the HFD + VA group compared with the HFD group (*P* < 0.05 and *P* < 0.01 for BW and visceral WAT mass, respectively). At P35, a similar pattern of significant changes in visceral WAT mass was observed, whereas no significant difference in BW was noted between groups. In addition, at P25, the BAT mass of pups was significantly (*P* < 0.05) reduced by maternal HFD consumption, but VA supplementation significantly (*P* < 0.01) increased the concentration to that in the NFD groups (Figure 1C). No significant difference in BAT mass was seen between groups at P14 and P35.

The adiposity index (AI) was calculated as the ratio of visceral WAT mass to BW (Figure 1D). At all 3 time points, the AI was dramatically (*P* < 0.001) higher in the HFD group than in the NFD groups. VA supplementation added to HFD significantly (*P* < 0.05) lowered the index, bringing the level back to that in the NFD groups. The data on BW, WAT mass, and BAT mass were presented in table format in our previous publication that addressed a different research question (31).

UCP-1 mRNA expression in BAT

UCP-1 is the protein responsible for adaptive thermogenesis in BAT. Its mRNA expression was measured using BAT samples from P25 and P35 (**Figure 2**). No significant difference was observed between the 4 groups at either P25 or P35, although pups in the HFD cohort showed a trend of decreased expression compared with those in the NFD cohort at both time points. The expression in pups from the HFD + VA group was significantly (*P* < 0.05) higher at P35 than at P25; such difference was not noted in the other groups.

Systemic and AT redox status
Oxidative stress.

At P25 and P35, the lipid peroxide concentration in WAT (**Figure 3**A) was significantly lower in the VA-supplemented groups than in their respective controls (*P* < 0.0001 for NFD + VA compared with NFD; *P* < 0.05 for HFD + VA compared with HFD); the concentration was significantly (*P* < 0.01) higher in the HFD + VA group than in the NFD + VA group, which was also noted at P14.

Owing to the inadequate amount of given BAT samples for TBAR analysis, data from the 3 time points were combined for statistical analysis to ensure an adequate power (Figure 3B). The NFD + VA group showed a significantly (*P* < 0.001) lower lipid peroxide concentration than the NFD group, whereas such effect of the VA supplementation was not observed in the HFD cohort.

Serum TBAR data from P14, P25, and P35 were also combined for analysis (Figure 3C). The lipid peroxide concentration in the HFD + VA group was significantly (*P* < 0.05) lower than in the HFD group, indicating a reduced systemic oxidative stress, whereas such difference was not noted in the NFD cohort.

AC.

The lipophilic AC of WAT at P25 and P35 was significantly (*P* < 0.05) higher in the HFD group than in the NFD group (**Figure 4**A). For BAT, a significantly (*P* < 0.05) higher lipophilic AC was noted in the HFD + VA group than in the NFD group (Figure 4B). No
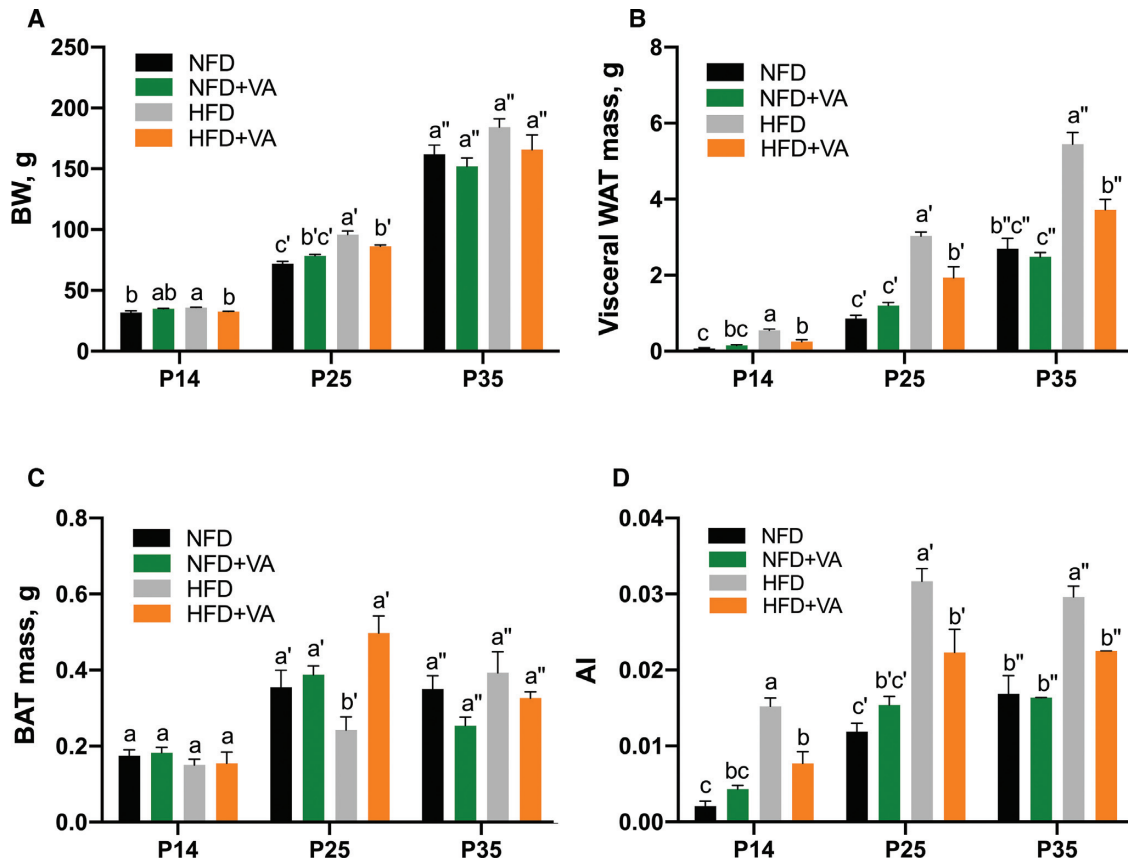
FIGURE 1 BWs (A), visceral WAT mass (B), BAT mass (C), and AI (ratio of visceral WAT mass to BW) (D) of rat pups at P14, P25, and P35. Bars show means ± SEMs, $n = 4$ or 3/group. One-factor ANOVA followed by Bonferroni post test was conducted at individual time points. Different letters at each time point indicate statistically significant differences, a > b > c, a' > b' >c', a" > b" > c", $P < 0.05$. AI, adiposity index; BAT, brown adipose tissue; BW, body weight; HFD, high-fat diet; NFD, normal-fat diet; P, postnatal day; VA, vitamin A; WAT, white adipose tissue.

significant difference in serum lipophilic AC was observed between groups (Figure 4C).

Correlation with VA status.
Correlation tests were run between measures of redox status as aforementioned and the concentration or total mass of VA in the same tissue [data published in (31)]. The mass of VA in WAT was found to have a significant positive correlation with the lipophilic AC of the tissue ($r = 0.360$, $P = 0.046$). In BAT, a positive trend between the mass of VA and the lipophilic AC was noted ($r = 0.495$, $P = 0.061$). A significant negative correlation was found between the concentration of VA and that of lipid peroxides in serum ($r = -0.679$; $P < 0.0001$).

Serum adiponectin and leptin at P25
As **Figure 5**A shows, a significantly ($P < 0.05$) higher serum adiponectin concentration was observed in the HFD + VA group than in the HFD group. Maternal HFD consumption significantly ($P < 0.05$) raised the serum leptin concentration (Figure 5B), whereas VA supplementation showed a trend toward reducing it.

Serum glucose and lipids at P25
Serum glucose concentration (**Figure 6**A) was significantly ($P < 0.05$) higher in the HFD group than in the NFD group; maternal VA supplementation to the HFD showed a trend toward decreasing the concentration. Figure 6B–E shows the profile of serum lipids. Pups in the HFD + VA group possessed a significantly ($P < 0.05$) lower serum total cholesterol concentration than those in the NFD + VA group. A significantly ($P < 0.01$) higher concentration of serum triglycerides was noted in the NFD + VA group than in the other groups. There was no significant difference in serum concentrations of LDL cholesterol or HDL cholesterol.

Serum inflammatory cytokines at P25
No significant differences in serum concentrations of IL-10, IL-6, KC/GRO, or TNF-α (**Figure 7**) between groups were noted, except that in the NFD cohort, maternal VA supplementation significantly ($P < 0.05$) increased the concentrations of IL-6 and IL-10. The concentrations were also significantly ($P < 0.05$) higher in the HFD group than in the NFD group.
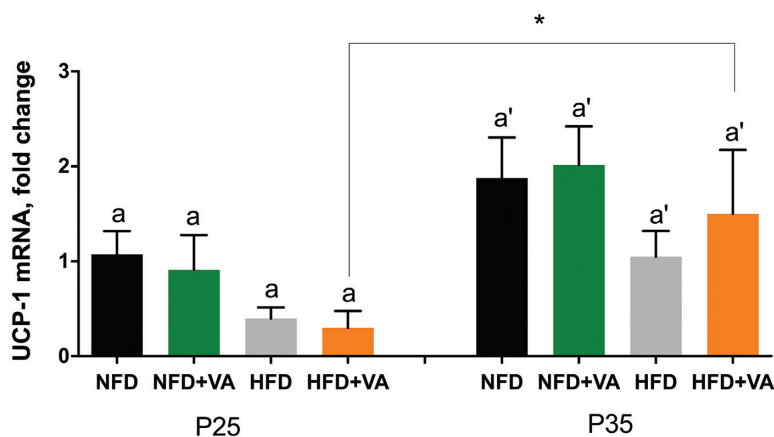
FIGURE 2 Brown adipose tissue UCP-1 mRNA expression in rat pups at P25 and P35. UCP-1 mRNA results were normalized to β-actin mRNA. Bars show means ± SEMs, n = 4 or 3/group. One-factor ANOVA followed by Bonferroni post test was conducted at individual time points. Groups sharing the same letter have no statistically significant difference. Student's t test was done to compare the expression in the same group between P25 and P35. *Statistically significant difference, $P < 0.05$. HFD, high-fat diet; NFD, normal-fat diet; P, postnatal day; UCP-1, uncoupling protein 1; VA, vitamin A.

Correlation among measures

Pearson correlation was conducted among measures of BW, visceral WAT mass, BAT mass, AI, glucose, lipids, adipokines, inflammatory cytokines, and measures of redox status at P25.

The lipophilic AC of WAT was positively correlated with BW ($r = 0.644$, $P = 0.007$), visceral WAT mass ($r = 0.737$, $P = 0.001$), and AI ($r = 0.735$, $P = 0.001$), whereas it was negatively correlated with serum total cholesterol ($r = -0.564$, $P = 0.036$) and LDL-cholesterol concentration ($r = -0.574$, $P = 0.032$). Serum glucose concentration was found to be positively correlated with BW ($r = 0.710$, $P = 0.007$), visceral WAT mass ($r = 0.665$, $P = 0.013$), AI ($r = 0.639$, $P = 0.019$), and serum HDL-cholesterol concentration ($r = 0.816$, $P = 0.001$). Serum IL-10 and IL-6 were positively correlated with several measures including serum glucose (with IL-10: $r = 0.815$, $P = 0.002$; with IL-6: $r = 0.664$, $P = 0.036$), triglycerides (with IL-10: $r = 0.765$, $P = 0.004$; with IL-6: $r = 0.640$, $P = 0.025$), and HDL cholesterol (with IL-10: $r = 0.821$, $P = 0.001$; with IL-6: $r = 0.679$, $P = 0.015$), and these 2 measures themselves were also strongly correlated ($r = 0.890$, $P < 0.0001$). There was a significant negative correlation between serum IL-6 and TNF-α concentrations ($r = -0.611$, $P = 0.035$).

Discussion

In the present study, we focused on neonatal and weanling rat offspring from mothers consuming diets meeting and exceeding recommendations for fat intake in humans. Although VA is known to be critical for many aspects of neonatal development, such as immunity and lung maturation, little is known about its role in neonatal AT development and associated metabolic conditions. To our knowledge, the current study is the first of its kind to show that VA supplementation to the HFD significantly reduced the BW and WAT mass but increased the BAT mass of the offspring, greatly attenuated their systemic and WAT oxidative

stress, and modulated the profile of serum adipokines and lipids to some extent.

Maternal HFD consumption dramatically increased the adiposity and compromised metabolic conditions of the offspring

It has been well established that maternal obesity or excessive gestational weight gain, including when induced by unhealthy dietary patterns, may increase the adiposity of the offspring as well as their risks of developing metabolic complications and/or diseases (8, 32). It was reported that neonates born to overweight/obese women were heavier than those of lean/average-weight women because of increased adiposity (33). In the current study, maternal HFD consumption during gestation did not increase the birth weight of rat pups, and the short duration of pregnant mothers receiving the HFD (gestational day 5–gestational day 21) may account for that. However, at P14, maternal HFD consumption during gestation and lactation had resulted in a dramatic increase in the BW and visceral WAT mass of the offspring: by 13.5% and 7-fold, respectively. The differences became more significant at P25.

Dietary patterns high in fat may influence local and systemic oxidative stress through WAT accrual and increased reactive oxygen species (ROS) generation (34, 35). A previous study conducted in a mouse model showed that maternal consumption of an HFD was associated with increases in oxidative stress that contributed to vascular dysfunction (36). A human study also indicated a significant association between maternal overweight or obesity and increased systemic oxidative stress levels in offspring (37). In the present study, although no significant difference in the lipid peroxide concentration was noted between the HFD and the NFD group, the HFD + VA group consistently showed significantly higher lipid peroxide concentrations in WAT than did the NFD + VA group. It may indicate that maternal HFD consumption can potentially compromise the effects of antioxidants in improving redox status. Our previously published data (31) indicated that the HFD + VA group possessed significantly more VA than the NFD + VA group,
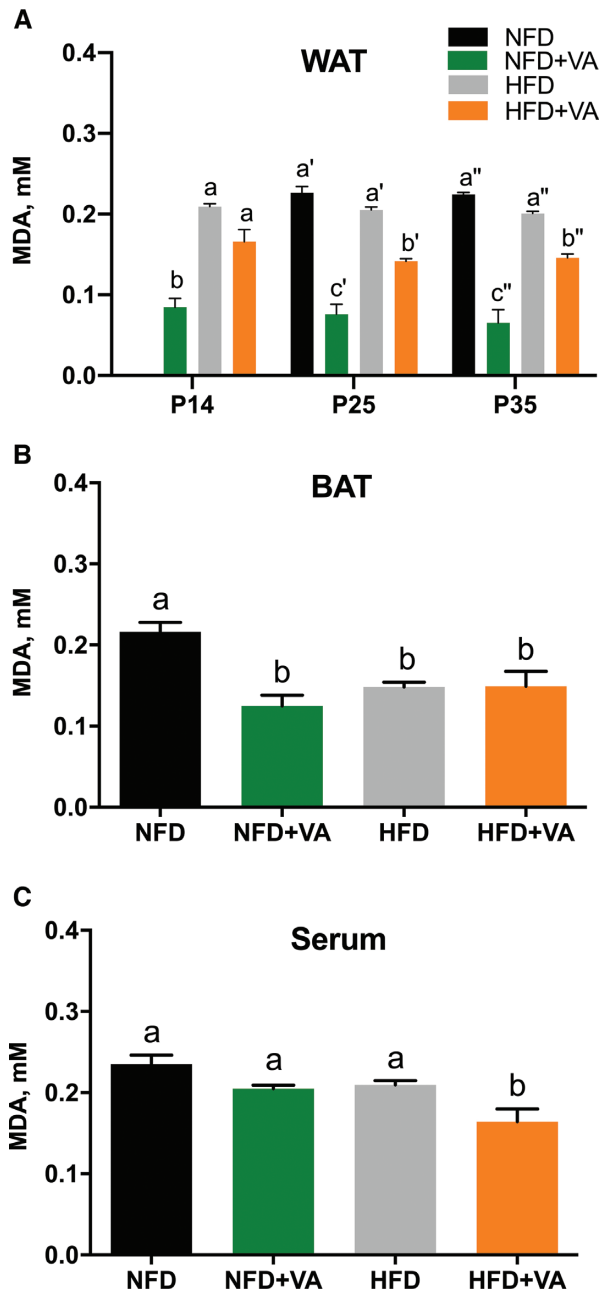
FIGURE 3 Concentrations of lipid peroxide in WAT of rat pups at P14, P25, and P35 (A), and in BAT (B) and serum (C) combining data from the 3 time points. Bars show means ± SEMs, $n = 4$ or 3/group for WAT and $n = 7$–11/group for BAT and serum. One-factor ANOVA followed by Bonferroni post test was conducted at individual time points. Different letters at each time point indicate statistically significant differences, a > b, a′ > b′ > c′, a″ > b″ > c″, $P < 0.05$. At P14, WAT samples in the NFD group were not adequate for analysis, and therefore the data are missing. BAT, brown adipose tissue; HFD, high-fat diet; MDA, malondialdehyde; NFD, normal-fat diet; P, postnatal day; VA, vitamin A; WAT, white adipose tissue.

FIGURE 4 Lipophilic antioxidant capacity of WAT of rat pups at P14, P25, and P35 (A), and of BAT (B) and serum (C) combining data from the 3 time points. Bars show means ± SEMs, $n = 4$ or 3/group for WAT and $n = 7$–11/group for BAT and serum. One-factor ANOVA followed by Bonferroni post test was conducted at individual time points. Different letters at each time point indicate statistically significant differences, a > b, a′ >b′ > c′, a″ > b″, $P < 0.05$. At P14, only 1 WAT sample in the NFD group was adequate for analysis, and therefore there is no error bar. BAT, brown adipose tissue; HFD, high-fat diet; NFD, normal-fat diet; P, postnatal day; VA, vitamin A; WAT, white adipose tissue.

supporting the potential role of WAT as a reservoir for dietary lipophilic antioxidants and micronutrients. This was further supported by the significantly positive correlation found between the lipophilic AC and the VA mass in the WAT. When sequestration of lipophilic antioxidants oc-
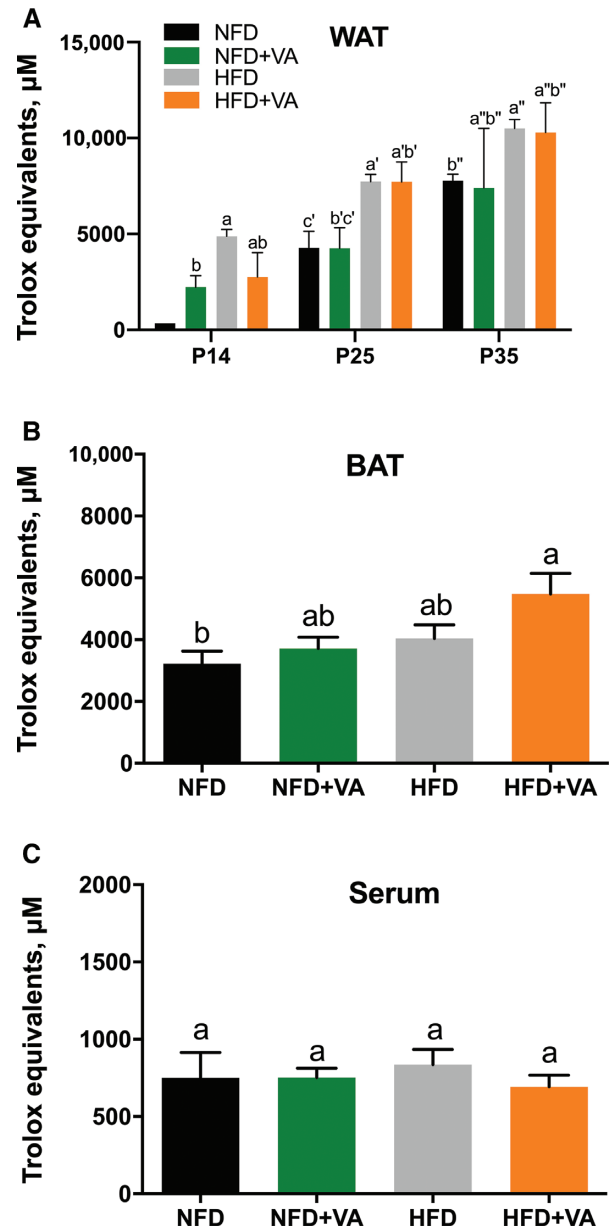
curs at a rate proportional to WAT mass, excess storage may induce a pro-oxidant state and thus an increased oxidative stress (38), as was exhibited in the HFD + VA group compared with the NFD + VA group.
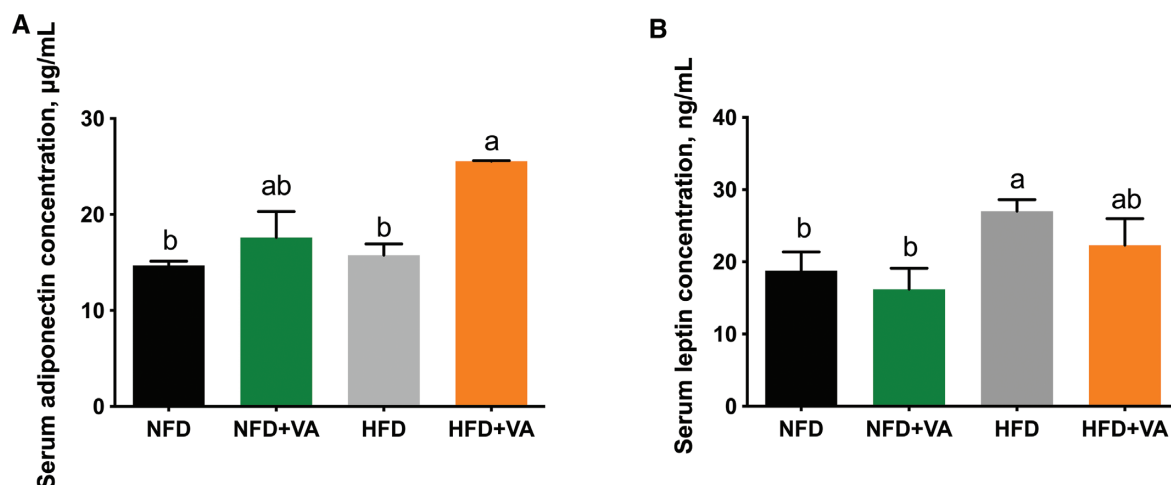
FIGURE 5 Concentrations of adiponectin (A) and leptin (B) in serum of rat pups at postnatal day 25. Bars show means ± SEMs, $n = 4$/group. Different letters indicate statistically significant differences, a > b, $P < 0.05$. HFD, high-fat diet; NFD, normal-fat diet; VA, vitamin A.

Significantly higher concentrations of serum glucose, leptin, IL-10, and IL-6 at P25 were also observed in pups of the HFD group than in those in the NFD group, indicating a perturbation by maternal HFD consumption of glucose homeostasis, adipokine balance, and inflammatory status. The result of serum glucose is consistent with a recent study conducted by Zhang et al. (39), in which hyperglycemia in mice offspring was reported to be induced by maternal HFD consumption through gestation and lactation. IL-6 and IL-10 are 2 inflammatory cytokines that have been reported to be higher in obese individuals in many studies (40, 41). The higher concentrations of these 2 cytokines associated with maternal HFD consumption observed in the current study may indicate a greater systemic inflammation caused by or related to excessive energy burden, higher WAT accrual, or increased oxidative stress. Serum IL-6 and IL-10 were also found to be correlated with multiple metabolic measures, including serum glucose, triglycerides, and HDL cholesterol, indicating their potentially important roles in metabolic health. Investigation of the correlation between maternal HFD consumption and the comprehensive inflammatory profile of the offspring is warranted.

VA supplementation during suckling and postweaning periods attenuated the metabolic disorders caused by the HFD consumption

The present study showed that VA supplementation greatly decreased the BW, visceral WAT mass, and the AI of pups receiving excessive fat, as the effects of VA supplementation or RA treatment have demonstrated in numerous studies with adult models (12, 13, 16–19, 22, 42–44). The neonatal stage is a critical period for WAT accumulation and development. In rat models, it is usually hard to find any WAT at birth (45). Around P14, visceral fat can be discerned and collected. Our results indicated a dramatic increase of visceral WAT mass from ∼0.07 g to 0.86 g between P14 and P25 in the NFD group and from ∼0.54 g to 3.03 g in the HFD group. In healthy human infants, the percentage of body fat doubles between birth and 6 wk of age (46). Nutritional interventions during the neonatal period are, therefore, critical in preventing or

attenuating potential over-growth or proliferation of adipocytes resulting from excessive energy intake. Our results showed that VA supplemented to the maternal HFD reduced the BW of the offspring by 10% measured at both P14 and P25 and decreased the visceral WAT mass by 54% measured at P14 and by 36% at P25. It is worthy of note that VA supplemented to the maternal NFD did not affect the BW or the adiposity of the offspring, indicating that VA would not interfere with the normal growth or AT development of pups receiving normal energy intakes. It is postulated that VA may only exert its regulatory effect on AT metabolism and development when excessive adiposity is present or in an obesogenic environment.

BAT, the main site for adaptive thermogenesis, was analyzed for multiple measures in the present study. Adaptive thermogenesis is the production of large amounts of heat through UCP-1 activation. The tissue is prominent in newborns and it makes up ∼5% of the body mass in human neonates and ∼0.5%–1% in rat neonates (47). In humans, BAT is gradually lost with age but may still contain beige adipocytes that can be potentially reactivated. Therefore, the tissue retains the capacity to play a significant role in energy balance and is a primary target organ in obesity prevention and management (48). Data from P25 in the present study showed that maternal consumption of HFD significantly reduced the mass of BAT, whereas VA supplementation restored the mass to that in NFD groups, thus potentially contributing to a higher level of adaptive thermogenesis and helping with weight control. This is in contrast to previous results in adults, which demonstrated that dietary VA supplementation or RA treatment either had no influence on BAT mass (12, 13, 18, 42, 49) or reduced the mass (19, 20, 43, 44). In adult models, dietary VA supplementation or RA treatment was also shown to induce the expression of UCP-1 in BAT (12–14, 17, 19, 44, 50), implying an increased thermogenic potential of BAT. Also in contrast to that, no change in BAT UCP-1 expression was observed in VA-supplemented pups in the current study. However, the expression in the HFD + VA group was significantly higher at P35 than at P25, whereas such a difference between the 2 time points was not noted in other groups. It is postulated that VA delivered through mother's milk may not be
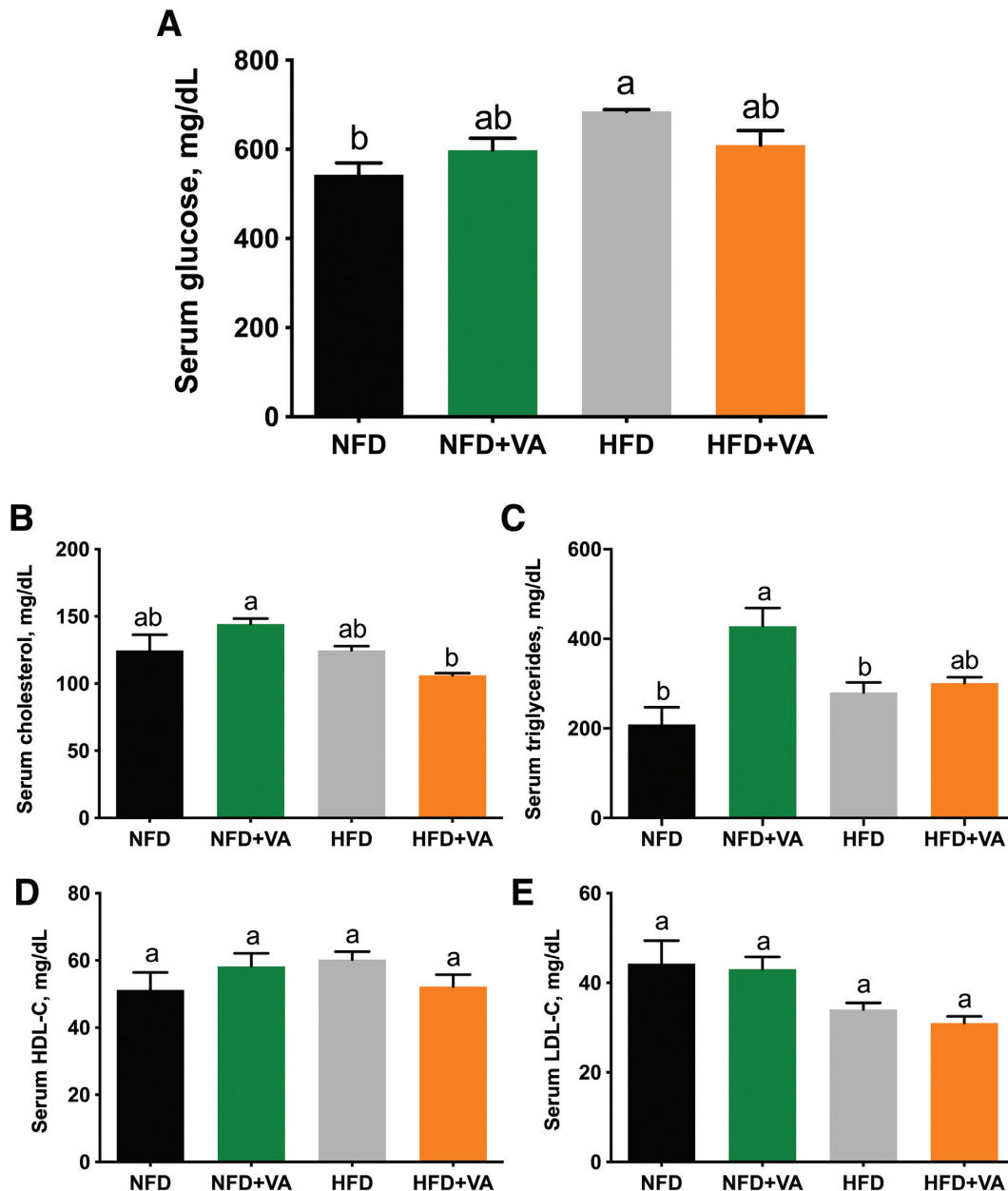
FIGURE 6 Concentrations of glucose (A), total cholesterol (B), triglycerides (C), HDL-C (D), and LDL-C (E) in serum of rat pups at postnatal day 25. Bars show means ± SEMs, $n = 4$/group. Different letters indicate statistically significant differences, a > b, $P < 0.05$. HDL-C, HDL cholesterol; HFD, high-fat diet; LDL-C, LDL cholesterol; NFD, normal-fat diet; VA, vitamin A.

able to exert as strong an effect as direct VA or RA treatment in regulating UCP-1 expression. However, the favorable results of maternal VA supplementation significantly reducing WAT mass but increasing BAT mass still warrant further investigation on VA's modulating role in AT development in the early life stage.

Although VA is not regarded as a traditional antioxidant, its effect on redox parameters has been explored in a few studies. Pasquali et al. (51) observed an increased lipid peroxidation level in the lung of adult rats that received VA treatments at multiple therapeutic doses for 28 d. In another study, rats received daily VA doses of 2500, 12,500, and

25,000 IU/kg during gestation and lactation; the treatments increased the serum concentration of lipid peroxide in male offspring, but decreased the concentration in female offspring (52). Despite the previous findings, the current study is the very first to explore the effects of maternal VA supplementation on both the AT and systemic redox status of offspring. VA supplementation was shown to improve both the systemic and WAT redox status in pups of dams consuming the HFD, as evidenced by the significantly lower lipid peroxide concentration in the HFD + VA group than in the HFD group. VA supplementation also lowered the lipid peroxide concentration in both WAT and BAT
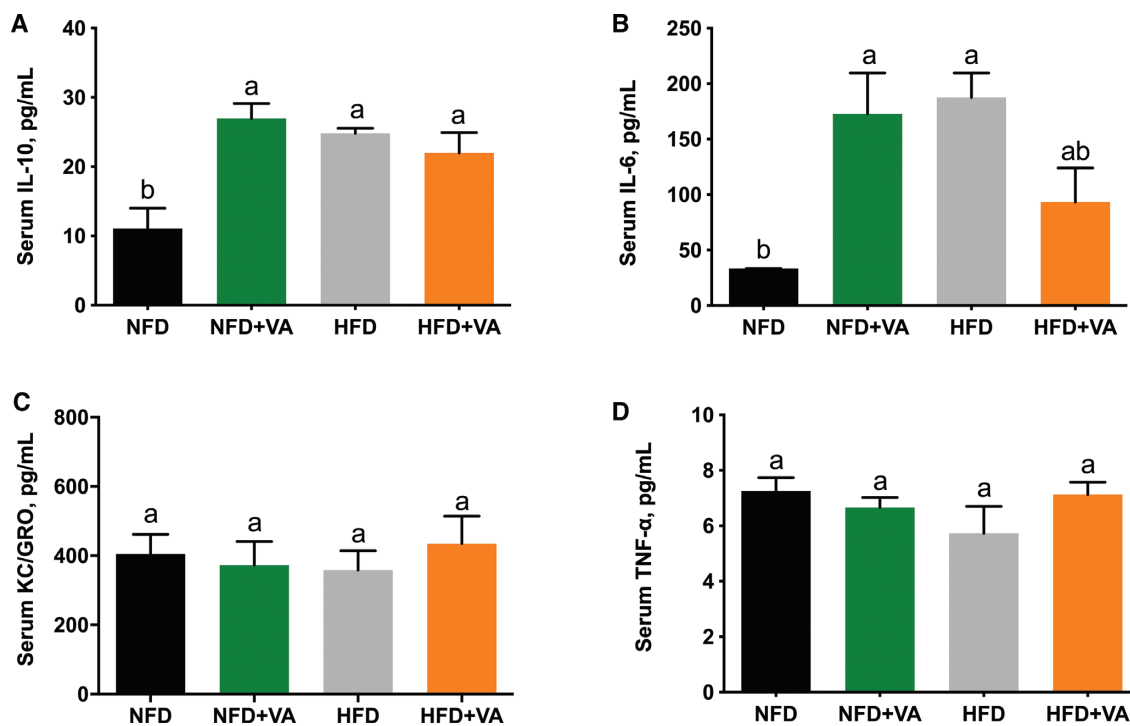
FIGURE 7 Concentrations of IL-10 (A), IL-6 (B), KC/GRO (C), and TNF-α (D) in serum of rat pups at postnatal day 25. Bars show means ± SEMs, $n = 4$/group. Different letters indicate statistically significant differences, a > b, $P < 0.05$. HFD, high-fat diet; KC/GRO, keratinocyte chemoattractant/growth-regulated oncogene; NFD, normal-fat diet; VA, vitamin A.

of pups of dams consuming the NFD. In addition, the lipophilic AC of the WAT was positively correlated with the VA mass in the tissue, whereas a strong negative correlation was noted between the serum concentration of VA and that of lipid peroxide. All of these findings support the role of VA supplementation in attenuating oxidative stress, which plays an important role in obesity and other chronic diseases. The differences between results of the present study and of previous ones may be related to the dose and administration mode of VA as well as the different tissues examined. It is worthy of note that VA supplementation reduced the oxidative stress of WAT but not BAT in pups of the HFD cohort. The results may be related to the greater contribution of WAT in the generation of ROS than BAT, especially in an obesogenic environment. It is known that ROS are mainly generated in the mitochondria and WAT possesses a larger number of mitochondria and subsequently generates greater oxidative stress owing to higher tissue mass than BAT (53).

Leptin and adiponectin are 2 major adipokines that play significant roles in obesity and metabolic health. Leptin concentrations are increased with higher adiposity, whereas adiponectin concentrations are downregulated in obesity. In the present study, maternal VA supplementation was found to increase pups' serum adiponectin concentration that was not altered by maternal HFD consumption and showed a trend toward reducing the leptin concentration that was significantly increased by maternal intake of HFD. Previous studies in adult rodent models showed that chronic dietary VA supplementation at the same amount as in the present study significantly suppressed serum leptin concentrations as well as leptin gene expression in WAT (17, 22). Acute

RA treatment was shown to downregulate the gene expression of both leptin and adiponectin in WAT in adult rats (23) as well as suppressing leptin gene expression in BAT (21). It should be noted that the current serum leptin and adiponectin data were only obtained from P25. By that time, rat pups in supplemented groups primarily received additional VA from mothers' milk. As compared with direct VA or RA administration as applied in aforementioned studies with adult models, the indirect route may have a weaker impact on the production and secretion of adipokines, as seen in the current study. However, maternal VA supplementation still showed a potential to restore the perturbed balance between leptin and adiponectin. Measuring the gene or protein expression of these 2 adipokines in future studies will provide more insights.

VA supplementation to the HFD was also found to influence several other metabolic measures of the offspring. It showed a trend toward decreasing the serum glucose concentration that was raised by maternal HFD intake and toward decreasing the serum cholesterol concentration. Interestingly, VA supplementation significantly increased the serum triglyceride concentration in the NFD cohort (NFD + VA compared with NFD). It was also reported in previous case studies that serum triglyceride concentration was elevated in patients receiving isotretinoin (13-cis-RA) for acne (54) or after a high-dose VA treatment to patients with pityriasis rubra pilaris (55). The mechanism is unknown. However, VA was shown to promote lipolysis in WAT (56) and, therefore, increase the release of free fatty acids and glycerol, which may resynthesize triglycerides in the liver, which are then packed into VLDLs and secreted into the circulation, leading to a higher serum concentration.

Concentrations of VA in supplemented and control diets

The current DRIs, including those for VA, were developed for the healthy general population. However, the percentage of adults aged ≥20 y with overweight, including obesity, has reached ∼70% ([57]). Therefore, it is of significance to consider developing DRIs for those populations or for reducing chronic diseases, as being considered by the National Academy of Medicine ([58]). Considering the high prevalence of maternal obesity as well as the upward trend in infant obesity, it is particularly important to look into modifying DRIs for overweight or obese lactating women.

In this study, the concentration of VA in the supplemented diets was 129 mg/kg, which was chosen based on previous studies ([13], [22]). On a BW basis, the mean daily VA intake of lactating rats in the supplemented groups (∼6000 μg/d) is much higher than the dietary recommendation for lactating mothers (1300 μg/d). As observed, the mean serum VA concentration in the supplemented groups was 5.5 μmol/L. This is much higher than the serum VA concentration regarded as normal, which is 0.7–1.75 μmol/L. However, no VA toxicity sign or symptom was noted, including depressed growth, occasional bleeding from the nose, or partial paralysis of the legs. An even higher dosage of VA supplementation was used in a previous study ([17]) and no toxicity was observed. In addition, at P35, the serum VA concentration in supplemented groups was significantly decreased as compared with that at P25, indicating a possible regulation mechanism to maintain VA homeostasis once pups start to receive VA directly from their diet. However, high amounts of VA supplementation should still be considered with caution to prevent toxicity, especially subtoxicity without clinical signs ([59]). This preclinical study lays the groundwork for future research to test doses of VA supplementation that are safer and are closer to dietary recommendations for humans, which will contribute to the establishment of VA DRIs for the overweight/obese population.

The control diets (NFD and HFD) contained 2.6 mg VA/kg, a concentration that is adequate to maintain a healthy pregnancy and fetal development, so that the VA status of control groups would mimic that of most pregnant women and healthy infants in the United States and other developed countries. The mean serum retinol concentration of pups in the control groups was ∼1.2 μmol/L, which is in the normal range. However, it should be pointed out that VA deficiency is a significant public health problem in many developing countries, particularly in South Asia and Africa, affecting a high percentage of pregnant women ([60]). The dietary intake of preformed VA or provitamin A carotenoids of these women is usually low. At the same time, a growing number of women of child-bearing age in these areas have become overweight or obese owing to unhealthy dietary patterns, creating the malnutrition problem encompassing both undernutrition and overnutrition ([2]). Therefore, future studies using a similar design to the present one but adopting a marginal or deficient amount of VA in control diets will be of translational meaning to help with solving public health problems in the developing world.

Strengths and limitations

Using limited tissue samples that could be obtained from young rats, this study evaluated their metabolic conditions via multiple measures, allowing for a comprehensive understanding of the effects of VA supplementation and an exploration on the correlation among measures.

Future studies are warranted to investigate the effects of maternal VA enrichment on genes or pathways involved in lipid metabolism, adipogenesis, angiogenesis, apoptosis, and WAT browning. Histological investigation on WAT and BAT will also provide valuable information. The adoption of multiple time points for tissue collection is another strength. P14, P25, and P35 are in the neonatal, weanling, and periadolescent phase for rats ([61]), respectively. The inclusion of these time points provided a dynamic picture of the BW change and AT development during early life. It also allowed for an exploration on the potentially differing effects of VA supplementation at different developmental stages. Data from P35 provided insights on how direct VA supplementation to rat pups may alter their metabolic conditions differently from supplementing the maternal diet.

Limitations should also be noted. Because of the nature of a neonatal rat model, the amounts of serum or tissue samples harvested were quite limited. As a result, several measures could only be conducted on samples from P25 and/or P35, or results from different time points needed to be combined for statistical analysis. Also, future studies with a larger sample size of maternal rats are needed to minimize the nonindependence of rat pups. However, the milk and serum VA concentration data confirmed that each individual rat pup in the VA-supplemented groups received significantly more VA and showed significantly elevated VA status. Pups started to consume food from P18 and were totally weaned from P25; therefore, the effects of VA supplementation were attributed to not only the indirect maternal supplementation but also direct supplementation to rat pups. Lastly, milk samples were analyzed for the concentration of VA but not other nutrients. Future research should explore whether maternal VA supplementation affects the mother's metabolism and changes the nutritional status of the milk, which may alter the BW gain and the adiposity of offspring as observed in the current study.

Conclusion and future directions

Results from the current study support the beneficial regulatory role of VA supplementation, especially through the enrichment of maternal diet, on the adiposity, metabolic profile, and potentially AT development of offspring from mothers consuming an HFD. It would be worthwhile to further explore supplementing maternal or infant obesogenic diets with VA or setting up a higher RDA of VA for obese lactating women as potential strategies in managing overweight/obesity in early life. Different amounts of VA and various administration routes should be studied to find the safest and optimal dose and mode. A longer study period extended to adulthood can also be considered to gain insights on how VA may affect the fetal programming effects of maternal obesity or obesogenic diet consumption.

Acknowledgments

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