Primming of Metabolic Dysfunctions by Prenatal Immune Activation in Mice: Relevance to Schizophrenia

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Schizophrenia is associated with increased risk for multiple metabolic abnormalities, including altered glucose homeostasis, type-2 diabetes, obesity, and cardiovascular disease. Some of the metabolic alterations can already exist in psychosis-prone subjects prior to the onset of chronic schizophrenic disease and pharmacotherapy, indicating that they may have a developmental origin. In the present study, we tested the hypothesis that metabolic alterations pertinent to schizophrenic disease can be primed by an environmental risk factor associated with the disorder, namely prenatal exposure to immune challenge. We used a well-established mouse model of prenatal immune challenge induced by maternal gestational treatment with poly(I:C) (="polyribonucleic-polyribocytidilic acid"), an analog of double-stranded RNA that stimulates a cytokine-associated viral-like acute phase response. Metabolic effects were studied using high-resolution computed tomography and fully automated indirect calorimetry system, along with an oral glucose tolerance test and plasma cytokine and corticosterone measurements. We found that prenatal immune activation caused altered glycemic regulation and abnormal ingestive behavior in perinatal and metabolic disturbances in patients with schizophrenia.4 However, accumulating evidence indicates that such abnormalities cannot solely be accounted for by APD exposure because they occur (albeit in a somewhat less severe form) already in drug-naive or minimally medicated first-episode patients.5–8 Thus, physiological and metabolic disturbances in psychosis-prone subjects seem to exist prior to the onset of chronic schizophrenic disease and may have a developmental origin.  

Introduction

It has been widely acknowledged that schizophrenia involves clinical features that relate to multiple central nervous system dysfunctions.1 In addition to the neuropathological and psychopathological manifestations, the disorder has been linked to numerous disturbances in basic physiological and metabolic functions, including insulin resistance, type-2 diabetes, obesity, and cardiovascular disease.2,3 It appears that chronic exposure to antipsychotic drugs (APDs), especially atypical APDs such as clozapine and olanzapine, may constitute one of the major contributing factors to at least some of the metabolic and cardiovascular malfunctions in patients with schizophrenia.4

Besides genetic factors, the susceptibility to physiological and metabolic disturbances are markedly influenced by environmental factors.9 Indeed, epidemiological studies in humans and experimental work in animals emphasize the critical role of the early-life environment in shaping postnatal physiological and metabolic functions. Stimulated by the seminal work of David Barker and colleagues,10 the concept of “early-life priming of adult disease” is now widely accepted. This concept refers to the phenomenon that a specific environmental factor acting during sensitive perinatal developmental periods can induce lifelong changes in physiological, metabolic, emotional, and behavioral functions.9,11

Prenatal exposure to infection is one of the environmental factors increasing the risk of schizophrenia and related psychotic disorders.12 Although the precise...
neuroimmunological mechanisms involved are still elusive, one prevalent hypothesis suggests that disruption of fetal neurodevelopmental processes by cytokine-associated inflammatory events predisposes the organism to long-lasting changes in subsequent brain and behavioral development, thereby increasing the risk of psychotic disturbances in later life.\textsuperscript{13,14} This hypothesis is supported by numerous studies in rodent models demonstrating altered fetal brain development and multiple long-term brain and behavioral abnormalities relevant to schizophrenia following prenatal exposure to infection and/or immune activation.\textsuperscript{14–16}

In keeping with the concept of “early-life priming of adult disease,”\textsuperscript{9–11} it is intriguing to speculate that in addition to its eminent neurodevelopmental impact, prenatal infection may also increase the risk to develop metabolic disturbances pertinent to schizophrenic disease. Human epidemiological research has thus far not been able to support or refute this hypothesis, and current attempts to model schizophrenia-relevant consequences of in utero exposure to infection/inflammation have largely neglected the potential impact of prenatal immune activation on long-term metabolic disturbances.\textsuperscript{14–16}

Therefore, the presented study tested the hypothesis that postnatal metabolic dysfunctions pertinent to schizophrenic disease can be primed by prenatal exposure to immune challenge. We used a well-established mouse model of prenatal immune activation induced by maternal gestational treatment with poly(I:C) (“polyriboinosine-polyribocytidilic acid”), a synthetic analog of double-stranded RNA that stimulates a cytokine-associated viral-like acute phase response.\textsuperscript{14–16} The mouse prenatal poly(I:C) model is characterized by solid face, construct, and predictive validities for schizophrenia-related pathology and has been widely used to study immune-mediated neurodevelopmental effects relevant to the disorder.\textsuperscript{14–16} Using this model, we explored the effects of prenatal immune activation on the postnatal development of physiological functions relevant to the metabolic syndrome in schizophrenia. This included measurements of gross body morphology and adiposity analysis\textsuperscript{2,3,5} as well as glucose homeostasis,\textsuperscript{6,7} energy expenditure,\textsuperscript{17} and ingestive behavior.\textsuperscript{18} These investigations were preceded by assessment of sensorimotor gating in the form of prepulse inhibition (PPI) of the acoustic startle reflex, a preattentive process known to be impaired in schizophrenia.\textsuperscript{16} The inclusion of PPI testing here served to verify the disrupting effects of prenatal immune challenge on adult sensorimotor gating\textsuperscript{14–16} and to ascertain possible associations between schizophrenia-relevant behavioral impairments and metabolic disturbances in offspring born to immune-challenged mothers. In addition, we measured peripheral levels of various cytokines and corticosterone (CORT) to examine whether the anticipated metabolic and behavioral changes in perinatally immune challenged offspring would be paralleled by persistent peripheral inflammation and/or changes in the output of the hypothalamus-pituitary-adrenal (HPA) axis. All investigations were conducted at 2 distinct maturational stages, namely in periadolescence and adulthood, so as to account for possible maturational effects in perinatally immune-challenged offspring relative to controls.

Materials and Methods

Animals

C57BL/6J mice bred in-house were used throughout the study. All animals were kept in a temperature and humidity-controlled (21 ± 1°C, 55 ± 5%) holding facility under a reversed light-dark cycle (lights off: 7:00 AM to 7:00 PM). They had ad libitum access to water and a standard low fat maintenance diet (extrudate chow KLIBA 3436, Provimi Kliba SA) with a caloric density of 13.1 MJ/kg (=3.128.9 kcal/kg) unless specified otherwise. All procedures were approved by the Cantonal Veterinary Office of Zurich and are in agreement with the principles of laboratory animal care in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 86–23, revised 1985). All efforts were made to minimize the number of animals used and their suffering.

Maternal Immune Activation During Pregnancy

Female mice were subjected to a timed mating procedure as described previously.\textsuperscript{19} Pregnant dams on gestation day 9 (GD 9) received either a single injection of poly(I:C) (potassium salt; Sigma-Aldrich) at a dose of 5 mg/kg (intravenous [i.v.]) or vehicle (sterile pyrogen-free 0.9% NaCl; i.v.) according to established protocols, which are fully described in the online supplementary information.

Testing of Offspring

Offspring from vehicle- or poly(I:C)-treated mothers were weaned and sexed on postnatal day (PND) 21. Littermates of the same sex were caged separately and maintained in groups of 2–4 animals per cage as described above. Control and poly(I:C) offspring were derived from multiple independent litters (6 per prenatal treatment condition) to minimize possible confounds from litter effects. All tests were conducted either when the offspring reached periadolescence, i.e., between PNDs 28–40 or when they reached adulthood (PNDs 70 onward). Periadolescent and adult stages were defined based on the gradual attainment of sexual maturity and age-specific behavioral discontinuities from younger to older animals.\textsuperscript{20} These 2 developmental stages approximately correspond to periods between 11 and 16 years and from 20 years onward, respectively, in humans.\textsuperscript{20} Only male offspring were included in all tests to avoid bias arising from sexual dimorphism.

Two independent cohorts were used for the behavioral and metabolic investigations conducted in periadolescence and adulthood to avoid potential confounding carryover effects from behavioral and/or metabolic testing during...
periadolescent brain maturation. For each maturational stage, we randomly selected 1 or 2 male offspring from one specific litter; a total of 12 distinct litters (6 prenatal poly(I:C) litters and 6 prenatal control litters) were used, yielding to a group size of 7–9 animals per treatment condition and maturational stage. Another 2 cohorts of animals were used for the cytokine and CORT analyses in periadolescence and adulthood. As for the behavioral and metabolic investigations, these animals stemmed from 12 independent litters (6 prenatal poly(I:C) litters and 6 prenatal control litters; see above). The sequence of testing and number of animals used in each test are described in the online supplementary information (online supplementary table 1).

**PPI of the Acoustic Startle Reflex**

The PPI test was conducted using 4 startle chambers for mice (San Diego Instruments) as described elsewhere. In the demonstration of PPI of the acoustic startle reflex, subjects were presented with a series of discrete trials comprising a mixture of 4 trial types. These included pulse-alone trials, prepulse-plus-pulse trials, prepulse-alone trials, and no-stimulus trials in which no discrete stimulus other than the constant background noise was presented. Overall, 3 different pulse intensities (100, 110, and 120 dB A) and 3 prepulse intensities (71, 77, and 83 dB A, corresponding to 6, 12, and 18 dB A above background of 65 dB A, respectively) were used. The PPI test session followed established protocols that are described in detail in the online supplementary information. For each of the 3 pulse intensities (100, 110, or 120 dB A), PPI was indexed by percent inhibition of the startle response obtained in the pulse-alone trials by the following expression: 100% \times \left(1 - \frac{\text{mean reactivity on prepulse-plus-pulse trials}}{\text{mean reactivity on pulse-alone trials}}\right)

**High-Resolution Microcomputed Tomography**

A high-resolution microcomputed tomography (μ-CT; La Theta LCT-100; Hitachi-Aloka Medical Ltd) was used to estimate whole body (nose-to-anus) adiposity and to differentiate subcutaneous from intraabdominal adipose tissue. The X-ray source tube voltage was set at 50 kV with a constant 1 mA current, with a pixel size of 0.10 × 0.10 mm and a coronal slice pitch of 1.00 mm according to established protocols. The animals were scanned under isoflurane anesthesia, which was induced in a small acrylic box using a flow of 400 ml/min O2 with 5% isoflurane and maintained in the scanner via a nose cone providing 100 ml/min 1% isoflurane. The animals were placed supine in the appropriate holder (inner diameter: 48 mm). First, a sagittal image of the entire animal was made to ensure proper placement in the holder and to set the scan area. Aloka software was used to estimate adipose tissue, bone, and the remainder (ie, lean mass) using differences in X-radiodensity (Housfield units) and to differentiate intraabdominal from subcutaneous adipose tissue using a built-in algorithm. In addition to the automated estimations, all coronal images were reevaluated by an experimenter blind to the treatment conditions and manually corrected when necessary to assure accuracy of tissue volume estimates. Stereoscopical reconstruction of CT scans was accomplished using Doctor-3D software (Able Software Corp.) and a standard 3D-rendering protocol. The following parameters were recorded and analyzed for each animal: Body weight (BW) (gram), body mass index (BMI; gram per square centimeter, where the length was defined as anal-nasal length), lean mass (gram), total fat (gram), visceral (=intraabdominal) fat (gram), subcutaneous fat (gram), and percent fat (=total fat/lean mass + total fat) × 100%.

**Indirect Calorimetry, Ingestive Behavior, and Activity**

A fully automated monitoring system including an open-circuit indirect calorimetry system (Phenomaster/Calo-System, TSE Systems GmbH) was used to measure circadian ingestive behavior, O2 consumption, and CO2 production. This system comprises a combination of sensitive eating and drinking sensors for automated online measurements and determines O2 consumption (milliliters per kilogram per hour), CO2 production (milliliters per kilogram per hour), and respiratory exchange rate (RER) (\(\frac{\text{VCO}_2}{\text{VO}_2}\) = CO2 production/O2 consumption) with the aid of high-speed gas sensing units. In addition, the animals’ activities were recorded with a photobeam-based activity monitoring system that detected vertical and horizontal movements. The system allowed simultaneous and continuous measurements of 12 animals kept individually in Eurostandard II home cages (67 × 207 × 140 mm, floor area 370 cm2). The cages were enclosed in an environmental control cabinet (TSE Systems GmbH) capable of maintaining specific temperature (30°C, corresponding to thermoneutrality), humidity (40% relative humidity), and illumination (60 and 0 Lux during light and dark phase, respectively) parameters. The latter were chosen to match our standard laboratory conditions (12/12-h light/dark cycle, with lights off from 7:00 AM to 7:00 PM). Data were collected on 2 consecutive days after an initial 24-hours acclimatization period. All animals had ad libitum access to water and food (extradate chow KLIBA 3436, Provi Mibla SA) during the initial acclimatization and subsequent recording phases. The following parameters were recorded: Fluid intake (milliliter), food intake (gram), total (horizontal and vertical) activity (meter), RER (\(\frac{\text{VCO}_2}{\text{VO}_2}\)), and heat production (kilocalorie per hour per kilogram). For each animal, the data collected on the active (dark) phase was averaged per animal and analyzed with the between-subjects factor of prenatal treatment.
Oral Glucose Tolerance Test

Glucose homeostasis was evaluated using an oral glucose tolerance test (oGTT) that measures the clearance of a standardized glucose load from the body.\(^{23}\) We used a fully oral GTT (instead of intragastric GTT) because we aimed to closely mimic the human condition, in which glucose is typically provided orally for these purposes.\(^{23}\) Blood samples were obtained at basal conditions (0 min, representing fasting glycemia) and at distinct times (+50, +60, +90, +120, and +150 min) after ingestion of a standardized glucose load (10 \(\mu\)g/g BW, of 20% wt/vol solution). The precise procedures used for the oGTT are fully described in the online supplementary information.

Plasma Cytokines and Corticosterone Measurements

The animals were killed by decapitation, and trunk blood was collected into Eppendorf tubes. The collected blood was allowed to clot at room temperature for 1 hour before centrifugation at 4000 rpm for 4 minutes at 4°C. The resulting plasma from each animal was subdivided to permit storage (\(-80°C\)) until later analyses of cytokines and CORT. Cytokine levels were measured using a multiplexed particle-based flow cytometric cytokine assay as described previously.\(^{24}\) Cytokines IL-1\(\beta\), IL-2, IL-6, IL-10, interferon-\(\gamma\) (IFN-\(\gamma\)), and tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) were purchased from R&D Systems (Wiesbaden-Nordenstadt). All procedures followed the manufacturer’s instructions. The analysis was conducted using a conventional flow cytometer (LSR II; BD Biosciences). The detection limits were 0.3 pg/ml for IL-1\(\beta\), 7.0 pg/ml for IL-2, 0.1 pg/ml for IL-6, 0.1 pg/ml for IL-10, 12.0 pg/ml for IFN-\(\gamma\), and 0.3 pg/ml for TNF-\(\alpha\). Plasma CORT levels were determined using a commercially available radioimmunoassay (CORT double antibody—\(^{125}\)I RIA Kit; MP Biomedicals) and were analyzed according to the manufacturer’s instructions.

Statistical Analyses

All data were analyzed using parametric ANOVA, followed by Fisher’s least significant difference post hoc tests whenever appropriate or by independent Student’s \(t\) tests (2-tailed). The precise statistical test used for each test is fully described in the online supplementary information. Statistical significance was set at \(P < .05\). All statistical analyses were performed using the statistical software StatView (version 5.0) implemented on a PC running the Windows XP operating system.

Results

Adult Onset of Sensorimotor Gating Deficiency Following Prenatal Immune Activation

Consistent with previous investigations in mice,\(^{14–16}\) we found that prenatal poly(I:C) treatment significantly reduced PPI scores (as indexed by percent inhibition) specifically in adult (figure 1b) but not periadolescent offspring (figure 1a), confirming that the disrupting effects of prenatal immune challenge on sensorimotor gating depend on postpubertal maturational processes. Prenatal poly(I:C) treatment did not significantly influence the startle reactivity as such (ie, the reactivity to pulse-alone stimuli) or prepulse-elicited reactivity (ie, the reactivity to prepulse-alone stimuli) neither in periadolescence nor in adulthood. The mean ± SEM startle and prepulse-elicited reactivities in periadolescent and adult offspring are summarized in online supplementary table 1.

Adult Onset of Increased Fat Deposition Following Prenatal Immune Activation

In keeping with the concept of prenatal priming of adult disease,\(^{9–11}\) we hypothesized that prenatal immune activation may significantly alter fat deposition in the postnatal life span. To test this, we assessed gross morphology (BW and length) and monitored fat deposition and lean mass using high-resolution \(\mu\)-CT in offspring born to immune-challenged mothers relative to controls. There were no significant group differences in any of these measures at the perinatal stage of development (figure 2a). At the adult stage, however, prenatally immune challenged offspring displayed a significant increase in visceral, subcutaneous, total fat, and percent fat compared with control offspring (figure 2b). These effects emerged in the absence of significant group differences in the measures of lean mass, BW, and BMI in adulthood (figure 2b).

Age-Dependent Alterations in Ingestive Behavior and Energy Balance Following Prenatal Immune Activation

We went on to explore whether offspring born to immune-challenged mothers may show signs of altered energy balance and abnormal ingestive behavior. For these purposes, we measured parameters of energy intake (fluid and food consumption) and energy expenditure (activity, RER, and heat production) using a fully automated indirect calorimetry system.

As shown in figure 3a, prenatally immune-challenged offspring showed a significant increase in fluid and food intake at periadolescence. Moreover, immune-challenged offspring also showed a significant enhancement in heat production at this maturational stage (figure 3a). Total (vertical and horizontal) activity in periadolescent offspring was not significantly affected by prenatal poly(I:C) exposure (figure 3a). The effects of prenatal immune activation on altered heat production persisted into adulthood, so that adult poly(I:C) offspring exhibited a significant increase in this measure relative to adult control offspring (figure 3b). Energy intake and expenditure were not significantly different between adult poly(I:C) and control offspring (figure 3b).
Glucose homeostasis was evaluated using an oral glucose tolerance test (oGTT), typically provided orally for these purposes. We measured parameters of energy intake (fluid and food consumption) and energy expenditure (activity, spontaneous locomotion, and food intake) using a direct calorimetry system. Prenatal immune activation may cause such alterations in periadolescence but not adult offspring born to poly(I:C)-exposed mothers. Fasting glycemia was similar in poly(I:C)-treated and control cohorts at either maturational stage investigated (figure 4).

Age-Dependent Changes in Peripheral Cytokine and Corticosterone Levels Following Prenatal Immune Activation

Finally, we were also interested to explore whether the metabolic and behavioral changes observed in prenatally immune challenged offspring would be paralleled by persistent alterations in peripheral cytokine secretion and/or changes in one of the bodily stress response systems, namely the HPA axis. As summarized in figure 5, we found that prenatal immune activation caused age-dependent changes in peripheral cytokine levels: periadolescent but not adult offspring born to poly(I:C)-exposed mothers displayed a significant reduction in IL-6 and TNF-α plasma levels (figure 5a). On the other hand, we revealed a marked reduction of IL-2 and IFN-γ specifically in adult but not periadolescent offspring exposed to prenatal immune activation (figure 5b). Prenatal immune activation did not significantly influence plasma IL-10 levels in periadolescence or adulthood, and plasma levels of IL-1β were below detection limit (0.3 pg/ml) for both ages investigated.

Prenatal immune activation also led to age-dependent alterations in basal CORT levels: periadolescent poly(I:C) offspring displayed a significant increase in plasma CORT contents (figure 5a), whereas CORT levels were not significantly different between poly(I:C)-exposed and control offspring at the adult stage of life.

Discussion

The present study tested the hypothesis that metabolic dysfunctions pertinent to schizophrenic disease can be primed by an environmental risk factor associated with the disorder, namely prenatal immune challenge. Using a well-established infection-based neurodevelopmental mouse model of schizophrenia-like pathology, we found that prenatal immune activation caused altered glycemic regulation and abnormal ingestive behavior in periadolescence and led to an adult onset of excess visceral and subcutaneous fat deposition. These effects were accompanied by age-dependent changes in peripheral secretion of proinflammatory (IL-6 and TNF-α) and T cell–related (IL-2 and IFN-γ) cytokines and by increased release of the stress hormone CORT in periadolescence.

Impaired glucose tolerance is one of the metabolic dysfunctions commonly observed in patients with schizophrenia, and as such, has often been used to index
Fig. 2. Adult onset of increased fat deposition following prenatal immune activation. The figure shows gross body morphology and adiposity analysis using high-resolution microcomputed tomography (μ-CT). (a) Offspring born to poly(I:C)-exposed or control mothers did not differ in gross body morphology or fat deposition at the periadolescent stage of development. N(control) = 7 and N(poly(I:C)) = 7 males; all values are means ± SEM. (b) Adult poly(I:C)-exposed offspring displayed a significant increase in visceral, subcutaneous, and total fat compared to adult control offspring and displayed a significant increase in percent fat (total fat/lean mass + total fat) × 100%). These effects emerged in the absence of significant group differences in the measures of lean mass, body weight, and body mass index. *P < .05, based on independent Student’s t tests (2-tailed). N(control) = 7 and N(poly(I:C)) = 7 males; all values are means ± SEM. (c) Stereoscopical reconstruction of CT scans derived from representative control and poly(I:C) offspring tested in periadolescence and adulthood. Subcutaneous and visceral fat is represented in yellow and pink color, respectively; overlays between subcutaneous and visceral fat highlights in orange color. Bone is depicted in white, whereas lean mass is excluded here. Note the marked increase in subcutaneous and visceral fat deposition in adult poly(I:C)-exposed offspring relative to adult control offspring.

A key finding presented herein is that prenatally impaired glucose tolerance can result from developmental exposure to a discrete environmental risk factor implicated in the etiology of schizophrenia.2–5 However, impaired glucose tolerance (albeit in a somewhat less severe form) has also been demonstrated in medication-naïve first-episode patients6,7 and can therefore not simply be explained by APD exposure alone. Rather,
such glycemic alterations may have a developmental aspect and may be primed by schizophrenia-related genetic and/or environmental risk factors. Using an experimental model system of prenatal immune challenge, our data show that impaired glucose tolerance can result from developmental exposure to a discrete environmental risk factor implicated in the etiology of schizophrenia. However, the effects of prenatal immune challenge on glucose tolerance were influenced by the age of the offspring and were overt only in periadolescence but not adulthood. This indicates that our model system may be incapable of mimicking altered glucose metabolism as observed in the adult (chronic) manifestation of schizophrenia but nevertheless emphasizes the impact of prenatal immune challenge on glycemic perturbations in the course of periadolescent maturation. In view of the latter, we consider it to be likely that exposure to additional factors such as APDs may be necessary to unmask latent priming effects of prenatal immune challenge on altered glucose metabolism across the adult life span. Notably, our study did not test this possibility directly, so that possible interactions between prenatal immune activation and APD exposure remain to be explored in future investigations. Another limitation of the present study is that we did not measure insulin levels in our cohorts of animals. Therefore, we cannot rule out the possibility that the lack of a significant treatment effect in the oGTT at adult age may reflect or may even be masked by potential differences in insulin levels.

A key finding presented herein is that prenatally immune-challenged offspring develop excess visceral and subcutaneous adiposity in adulthood (figure 2). Increased visceral and subcutaneous fat deposition has also been repeatedly documented in patients with schizophrenia. The adiposity-promoting effects of APDs seem to be critical in mediating excessive fat deposition and obesity in schizophrenia. However, initial evidence shows that increased adiposity can already exist in drug-naïve first-episode patients, suggesting that individuals who later go on to develop schizophrenic disease may have a metabolic predisposition that facilitates the accumulation of visceral and subcutaneous fat. Our findings are consistent with this interpretation and highlight that prenatal immune activation may be a relevant factor. 

Fig. 3. Age-dependent alterations in ingestive behavior and energy balance following prenatal immune activation. (a) Peripubertal offspring born to poly(I:C)-exposed mothers displayed a significant increase in heat production, fluid (water) intake, and food (standard lab chow) intake relative to adult control offspring. *P < .05 and **P < .01, based on independent Student’s t tests (2-tailed). N(control) = 7 and N(poly(I:C)) = 7 males; all values are means ± SEM. (b) Adult poly(I:C) offspring showed a significant increase in heat production relative to adult controls. *P < .05, based on independent Student’s t test (2-tailed). N(control) = 7 and N(poly(I:C)) = 7 males; all values are means ± SEM.

Prenatal Infection and Metabolic Dysfunctions
Fig. 4. Impaired glucose tolerance in periadolescence following prenatal immune activation. Glucose homeostasis was evaluated using an oral glucose tolerance test, in which blood samples were obtained at basal conditions (+0 min, representing fasting glycemia) and at distinct times (+30, +60, +90, +120, and +150 min) after ingestion of a standardized glucose load. (a) Periadolescent offspring born to poly(I:C)-exposed mothers displayed impaired glucose clearance as evident in the significant increase in glycemia at 30 min after glucose ingestion; **$P<.01$, based on Fischer’s post hoc tests conducted at each individual time point following the presence of a significant interaction between prenatal treatment and postglucose interval ($F_{2,30}=5.43, P<.001$) in the 2 × 6 (prenatal treatment × postglucose interval) repeated-measure ANOVA. There was also a significant group difference in terms of integrated glycemia as indexed by the area under the curve of blood glucose levels from +0 to +150 min postglucose ingestion; *$P<.05$, based on independent Student’s $t$ test (2-tailed). $N$(control) = 6 and $N$(poly(I:C)) = 6 males; all values are means ± SEM. (b) Adult poly(I:C)-exposed and control offspring did not significantly differ in any of the parameters indexing glucose homeostasis. $N$(control) = 7 and $N$(poly(I:C)) = 7 males; all values are means ± SEM.

predisposition factor for metabolic disturbances leading to increased adiposity in adulthood. However, it should be noted that adult poly(I:C)-exposed offspring cannot be considered to be obese, despite the fact that they showed a significant increase in visceral and subcutaneous fat at adult age. The amount of total (visceral and subcutaneous) fat in adult poly(I:C)-exposed subjects was ~6 g, whereas the amount of total fat in adult control subjects was ~4 g (figure 2). The increase of fat in adult poly(I:C)-exposed subjects relative to adult controls emerged against a background of ~20 g lean mass, the latter of which was not significantly different between the 2 groups (figure 2). Furthermore, adult poly(I:C)-exposed and control offspring did not differ with respect to BMI, which is typically taken as the standard measure to index obesity. Hence, the effects of prenatal immune activation on adult fat deposition are subtle (but significant) but do not reflect obesity. Interestingly, the subtle effects identified in our model system readily correspond to the magnitude of increased fat deposition in drug-naive or minimally medicated first-episode patients.17,18,25,28 Our findings virtually exclude the possibility that the latter contributed to the adiposity phenotype observed here because if anything, offspring born to poly(I:C)-exposed mothers displayed significantly increased energy expenditure (as indexed by indirect calorimetry), an effect that was similarly present both in periadolescence and adulthood (figure 3). On the other
hand, it seems feasible that the development of increased fat deposition in prenatally immune-challenged animals may be related to the presence of abnormal ingestive behavior: We found that food and fluid consumption was significantly increased in poly(I:C)-exposed offspring relative to control offspring, especially in periadolescence. It is therefore reasonable to assume that the initial changes in ingestive behavior facilitated the accumulation of fat across the periadolescent to the early adult stage of development, so that the adiposity phenotype becomes evident only once the offspring reach early adulthood.

The mechanisms responsible for the prenatal infection-induced changes in ingestive behavior remain essentially unknown. However, it is interesting to note that increased food and fluid intake in periadolescent poly(I:C) offspring temporally coincided with a significant reduction in peripheral levels of the proinflammatory cytokines IL-6 and TNF-α. Besides their essential roles in the inflammatory response, both cytokines affect ingestive behavior, presumably by acting directly on the brain. In particular, IL-6 and TNF-α are well known to inhibit food and fluid intake, as exemplified by the reduction in intake during infections and associated sickness behavior. In view of these effects, reduced peripheral production of proinflammatory cytokines such as IL-6 and TNF-α may readily contribute to the increase in food and fluid intake in periadolescent poly(I:C) offspring. Additional circumstantial support for this suggestion is derived from experiments in mice showing that genetic IL-6 or TNF-α receptor I deficiency increases food intake and facilitates adult-onset obesity.

Our observation of reduced peripheral IL-6 and TNF-α levels in periadolescent poly(I:C) offspring suggests that a single exposure to viral-like immune challenge in early/middle gestation does not lead to persistent systemic inflammation in the offspring, at least under basal conditions. This contrasts the long-term immune effects of more severe prenatal immune activation
regimes such as chronic maternal inflammation models in rats, which have been shown to induce persistent elevation of peripheral proinflammatory cytokines in the offspring. Furthermore, our findings do not parallel the numerous reports of elevated proinflammatory cytokine secretion in schizophrenic patients, suggesting that acute prenatal viral-like immune challenge is insufficient to induce such immune abnormalities in the postnatal lifespan. It has been suggested that the emergence of persistent low-grade inflammation in schizophrenic patients may be related to (or even induced by) genetic susceptibility factors such as polymorphisms in proinflammatory cytokine genes and promoter regions, so that our purely environmental model would not be able to mimic this particular immunological aspect of schizophrenia.

Interestingly, the decrease in peripheral proinflammatory cytokine secretion observed in periadolescent poly(I:C) offspring coincided with enhanced CORT release. The latter effect indicates that periadolescent offspring born to immune-challenged mothers display abnormally enhanced activity of the HPA stress response axis even under basal conditions. Increased (basal and stress-induced) CORT release has been widely documented in patients with schizophrenia, and as such has been taken as evidence for abnormal stress receptiveness and/or responsiveness in affected individuals. Besides its broad metabolic functions, CORT is an essential part of the homeostatic control of the immune system, where it potently suppresses various immune functions in general and inflammatory responses in particular. With regards to the latter, CORT exerts potent anti-inflammatory properties by suppressing the production and/or secretion of proinflammatory cytokines. It is therefore feasible that the reduction in peripheral proinflammatory cytokines in periadolescent poly(I:C) offspring may be linked to the presence of increased basal CORT levels.

Our study also shows that adult offspring born to poly(I:C)-exposed mothers display a marked reduction in plasma IL-2 and IFN-γ levels. IL-2 and IFN-γ are both produced primarily by the T-helper (Th1) subset of Th cells, so that reduced secretion of these cytokines may reflect blunted production of specific Th1 cytokines. As reviewed elsewhere, at least a subset of schizophrenic patients show impaired Th1-related immune functions such as blunted peripheral IL-2 and IFN-γ production, and this has been interpreted as a sign of imbalanced Th1/Th2 immune functions in affected individuals with a relative shift toward the Th2 system. It has been hypothesized that such imbalances may result, at least in part, from abnormal development of T-cell networks triggered by early (prenatal) life exposure to infection. Our results are the first to support this hypothesis using a well-established model of prenatal viral-like immune activation in mice that has previously been shown to capture a wide variety of schizophrenia-relevant brain and behavioral abnormalities.

In conclusion, our data provide novel evidence that prenatal immune activation is a significant environmental factor in the development of postnatal metabolic and immunological abnormalities. Some of these abnormalities, including increased visceral fat deposition and blunted Th1-related cytokine secretion, are reminiscent of metabolic and immunological changes observed in adult patients with schizophrenia, indicating that prenatal viral-like immune activation may play a significant etiological role in the development of such dysfunctions. In contrast, the lack of persistent peripheral inflammation and glycemic abnormalities in adult offspring born to poly(I:C)-exposed mothers may be taken as experimental evidence to suggest that prenatal viral-like immune activation may only play a minor role in the precipitation of long-term proinflammatory profiles and altered glucose metabolism. It thus appears that distinct schizophrenia-relevant metabolic and immunological abnormalities can be primed by prenatal viral-like immune activation, but this environmental insult is unlikely to mediate the full spectrum of metabolic and immunological changes pertinent to chronic schizophrenic disease.

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Supplementary Material
Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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