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Original Research Article

Postpartum levels of 8-iso-prostaglandin $F_{2\alpha}$ in plasma and milk phospholipid fractions as biomarker of oxidative stress in first-lactating dairy cows

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ABSTRACT

F_2-isoprostanes such as 8-iso-prostaglandin F2<alpha> (8-iso-$PGF_{2\alpha}$) are formed by free radical-catalyzed mechanisms from membrane phospholipids and from low density lipoproteins through peroxidation of arachidonic acid. Esterified 8-iso-$PGF_{2\alpha}$ is cleaved by phospholipases, circulates in blood and is excreted as putatively harmful oxidatively modified lipid via the kidney into urine. In this study we demonstrate that 8-iso-$PGF_{2\alpha}$ concentrations in plasma samples from heifers are higher ($p < 0.005$) compared to those from first-lactating dairy cows at 71 days postpartum. Furthermore, plasma 8-iso-$PGF_{2\alpha}$ concentrations vary with ovarian activity and differ in response to luteolytic initiation as well as activation of the hypothalamic–pituitary–gonadal axis between heifers and first-lactating cows. Sustainable concentrations of 8-iso-$PGF_{2\alpha}$ (50–150 pg/ml) are detectable in the phospholipid fraction of milk, suggesting milk as an additional excretion route for 8-isoprostanes. Plasma levels largely paralleled levels in milk ($p < 0.001$). Plasma phospholipid 8-iso-$PGF_{2\alpha}$ concentrations in cyclic cows decreased ($p < 0.05$) from day 38 to day 71 postpartum, whereas milk phospholipid 8-iso-$PGF_{2\alpha}$ rather increased ($p < 0.05$). Cyclic cows tend to have higher 8-isoprostane levels compared to acyclic animals. In contrast to lipohydroperoxides, concentration of 8-iso-$PGF_{2\alpha}$ were not correlated with milk yield ($p > 0.05$). Our data indicate 8-iso-$PGF_{2\alpha}$ may be a novel biomarker of oxidative stress in dairy cow, which is detectable in blood as well as in milk.

Introduction

Reproductive dysfunction observed in first-lactating cows has been attributed to metabolic adaptation which is needed to ensure energy demand for lactation. This is especially critical during the postpartum period of negative energy balance a time period where fertilization is highly desirable. The underlying mechanisms of reproductive dysfunction in dairy cows are not understood [1,2].

In dairy cow energy output via milk production has been shown to be a linear function of metabolizable energy intake [3]. Ovarian function needs to be maintained even during periods of high milk production as long as metabolizable energy input is adequate. This energy input is correlated with the rate of heat production, which is a measure of oxidative metabolism and whole body oxygen consumption rate in cows [4]. An increase in oxidative metabolism is likely to be associated with a higher production of reactive oxygen metabolites [5]. Some reactive metabolites escape from endogenous defense systems. Overproduction of oxidants that overwhelms the cellular antioxidant capacity is called oxidative stress. High-performing dairy cows are prone to oxidative stress due to increased metabolic activity by concurrent limited defense capacity [6]. Reactive oxidative metabolites are not only harmful factors, but may also serve as informative biomarkers. Examples are peroxidation products of unsaturated lipids and the formation of F_2-isoprostanes (also denoted as 8-isoprostanes) including 8-iso-$PGF_{2\alpha}$ [7–9]. Reactive oxygen metabolites may also serve as precursors of enzymatic reactions, e.g. prostaglandin synthesis by cyclooxygenases [10]. Thus, the boundary of oxidative stress and oxidative strain is not precisely defined [7].

8-iso-$PGF_{2\alpha}$ is formed via a non-enzymatic mechanism involving the free radical initiated peroxidation of arachidonic acid [8,11]. In a number of cell types the activity of prostaglandin-H_2 synthase (PTGS2) is a source of 8-iso-$PGF_{2\alpha}$ [12]. An increase in expression of PTGS2 in bovine ovarian follicles starts about 16 h subsequently after preovulatory gonadotropin surges [13,14]. The surge is triggered endogenously by elevated hypophyseal gonadotropin

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Table 1
Nutritional composition of total mixed ration.

Item	Heifers	Cow[a]	Cow[b]
Ingredient [g/kg of DM]			
Corn silage		455	446
Grass silage	950	262	228
Straw		10	
Hay	45		67
Concentrate MLF2000[c]			180
Concentrate mixture[d]		266	49
Dried beet pulp			23
Minerals	5[e]	7[e]	7[e]
NE_L, MJ/kg of DM	5.6	7.1	6.9

[a] Cow Steinhagen.
[b] Cow Dummerstorf.
[c] Concentrate MLF2000 (Vollkraft, Güstrow, Germany) provided the following: 33% extracted soybean meal, 20% corn, 17% wheat gluten, 13% wheat, 8% extracted rapeseed meal, 5% sugar-beet pulp, 2% sodium hydrogen carbonate, 1.3% calcium carbonate, 0.2% sodium chloride, 8.0 MJ of NE_L/kg of DM, and 204 g of utilizable protein/kg of DM.
[d] Concentrate mixture: molasses (0.9%), dry beet pulp (3.1%), grain maize silage (2.1%), wheat (moist grain) (4.9%), soy bean meal (2.6%), extracted rapeseed meal (2.6%), dried stillage (wheat) (4.02%) and urea (0.1%).
[e] Rinderstolz 9522 (Salvana) provided the following: 92% crude ash, 20% calcium, 5% phosphorus, 6% magnesium, 8% sodium, and vitamins.

secretion or via injection of GnRH after regression of the corpus luteum to synchronize ovarian follicle development. Since 8-iso-$PGF_{2\alpha}$ is (at least in part) synthesized enzymatically, which would indicate an active HPG axis, we hypothesized that 8-iso-$PGF_{2\alpha}$ concentrations in cyclic and noncyclic dairy cows as well as between heifers and cows might be different.

To test these hypotheses we first tested whether 8-iso-$PGF_{2\alpha}$ levels in cyclic heifers and cyclic dairy cows respond differently to GnRH induction. Secondly, we compared plasma and milk 8-iso-$PGF_{2\alpha}$ levels from phospholipids and whole plasma of cyclic and acyclic dairy cows at different days postpartum. Finally, we compared lipohydroperoxide and 8-iso-$PGF_{2\alpha}$ levels from phospholipids according to the milk production in cows.

Materials and methods

Animals

First lactating German Holstein cows ($n = 20$) and heifers ($n = 12$) (16–19 month old) were housed at the dairy cattle research farm of the Leibniz Institute for Farm Animal Biology in Dummerstorf and first-lactating high-performance German Holstein cows ($n = 52$) at a commercial dairy cattle farm (Griepentrog KG) in Steinhagen. All animals were offered a total mixed ration (TMR) (Table 1) with an intake ad libitum consisting of grass and corn silage. Concentrate containing minerals and vitamins were added to TMR allowing growth of heifers about 450 ± 50 g daily. Lactating cows were fed concentrates with minerals and vitamins according to dairy energy output. Subsequent milking (twice per day in Dummerstorf, thrice in Steinhagen), residual milk was sampled (10 ml) before animals were fed TMR. At the same time (when not otherwise stated), blood was drawn by coccygeal puncture (for assay conditions, see below). Milk yield, fat corrected milk (FCM) 4% = milk (kg/day) × (0.4 + milk fat % × 0.15) and ingredients were automatically recorded by infrared spectroscopy. Animal handling and treatments were approved by the governmental Committee Mecklenburg-Vorpommern on Animal Use and Care.

Ovarian cycle synchronization

Ovarian activity was assessed by monitoring estrous behavior, determination of plasma and milk progesterone levels, and ovarian ultrasonographic examination as described previously [15–17]. Ultrasonographic examinations were performed by B-mode Doppler ultrasound scanner (Micromaxx, Sonosite) equipped with a 7.5-MHz, linear-array intrarectal transducer according to a recently described technique [17]. The presence of a functional corpus luteum was taken as an index of cycling heifers and cows. Corpora lutea were regressed by injection of a prostaglandin $F_{2\alpha}$ analog (Cloprostenol, 20 mg, i.m.) subsequent 9–13 days post estrus. Regression of corpora lutea was monitored by ultrasonography and the decline in progesterone. A dominant follicle development was also monitored by B-mode and color Doppler ultrasound (increasing diameter and vascularization) and the preovulatory process was induced by injection an analog (Depherelin, 100 μg, i.m.) of gonadotropin releasing hormone (GnRH) 54 h after cloprostenol administration.

Assays

Progesterone (P4)

Progesterone concentration was measured in 5 μl plasma by a direct single purified rabbit antibody by RIA [17,18]. This antibody cross-reacts 12.5%, 6% and 0.9% to 5β-dihydroprogesterone, other progestagens/progestins and steroids, respectively. The sensitivity of the assay was 0.4 ng/ml. The intra- and interassay coefficients of variation for progesterone were 9% and 15%.

Lipohydroperoxide (LOOH)

Plasma was prepared by centrifugation of coccygeal blood (at 4 °C, 4000 × *g*, 10 min, 6 mM EDTA, pH 7.2) and stored on ice. Immediately after sampling, butylated hydroxytoluene (to 220 μM) was added as antioxidant, sucrose (to 0.4 M) to stabilize lipoprotein particles and samples were stored at −80 °C. Milk was collected on ice and centrifuged (300 × *g*, 10 min, 4 °C) to obtain cell-free milk samples and stored at −80 °C. Phospholipids from plasma and milk were extracted using the kit for LOOH determination (#705002, Cayman Chem Comp, Ann Arbor, MJ). Chloroform and methanol have been freshly deoxygenated. Two-hundred μl samples (plasma or milk) were deproteinized by adding equal volumes of Extract R (LOOH kit component) in saturated methanol and vortexed. Then 1 ml of chloroform was added and phospholipids were extracted via centrifugation. LOOH was assayed in the organic phase within 1 month after sample collection as described earlier [6,18].

8-iso-Prostaglandin F2α (8-iso-$PGF_{2\alpha}$)

For assay of 8-iso-$PGF_{2\alpha}$, the organic phase was evaporated within 10 min under nitrogen. The residuals were reconstituted by buffer provided with the 8-isoprostane EIA kit (Cayman Chem Comp, Ann Arbor, MJ) and measured as described previously [19]. In order to estimate extraction efficiency for the phospholipid extraction procedure (see above), defined concentrations of 8-iso-$PGF_{2\alpha}$ standards were spiked into the extracted samples. The recovery efficiency was 97 ± 2%. The manufacturer described the cross-reactivity of the assay with 8-iso-$PGF_{3\alpha}$ and 2,3-dinor-8-iso-$PGF_{2\alpha}$ to be 20.4% and 4.0%, with other 8-isoprostanes less than 2%, respectively. The detection limit from phospholipid plasma and milk samples were 5 pg/ml and the coefficient of intra- and interassay variation at levels ≤10 pg/ml 8-iso-$PGF_{2\alpha}$ about 16%; at levels of 50–200 pg/ml below 10%.

Statistical analysis

Results are presented as means and standard error of the mean (SEM) when not otherwise stated. To determine which means differed, comparisons between means were accomplished by ANOVA (one-way ANOVA) and t-test, multiple comparisons by ANOVA and