

Method Article

Effect of Early Dietary Interventions on Energy Expenditure and Nutrient Oxidation in Newborn Intrauterine Restricted Piglets: A Pilot Study

UNDER PEER REVIEW

ABSTRACT

Aims: To evaluate the feasibility of determining energy expenditure and nutrient oxidation using respiration chambers for newborn **intrauterine growth-restricted** (IUGR) piglets while testing two different dietary interventions (DI).

Study design: A 2x4 factorial design was used in this study.

Place and Duration of Study: Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark, between September and October 2016.

Methodology: Two different dietary interventions were used for IUGR piglets, Glucose (Glu) injected **subcutaneously and** a tube-fed dosage of porcine colostrum at two different levels of temperature; 22°C (T22) and 36°C (T36) compared with a placebo (P) treatment (water) of both IUGR and normal (Norm) piglets. Forty-eight piglets in total with 6 piglets per **group** were used. Piglets were weighed prior to insertion into respiratory chambers, where oxygen (O₂), carbon dioxide (CO₂) and methane (CH₄) emissions were measured in 1h intervals over a 4h period. Respiratory quotients (RQ) and heat increment (HI) were calculated based on O₂, CO₂, and CH₄ values.

Results: Due to too few animals per group no statistical analysis was performed. Not until between 1h to 2h after DI in T36 IUGR-Glu and -Col piglets displayed numerically greater HI/kg BW compared with IUGR-P (12.7% and 11.3% vs. 7.5%), whereas in T22 IUGR-Col and -P displayed similar increases which were numerically greater than IUGR-Glu piglets (13.0% and 12.8% vs. 9.1%). However, 2h and 3h after DI, respectively, only IUGR-Col and Norm-P in T22 and IUGR-Glu in T36 displayed HI/kg BW (0.56, 2.04 and 0.81%). In the period from the 1h to 2h after DI in T36 RQ changed from 0.34-0.57, 0.28-0.55, 0.32-0.58, 0.52-0.71 for IUGR-P, -Glu, -Col and Norm-P piglets, respectively.

Conclusion: Determining energy expenditure and nutrient oxidation in newborn IUGR piglets using respiration chambers is associated with great technical difficulties leading to results that should be carefully interpreted. However, positive HI **was** observed when piglets **were** given DI after 1h in all groups.

Keywords: energy expenditure, nutrient oxidation, intra-uterine growth restriction, dietary intervention

1. INTRODUCTION

Due to **the** selection **of** large litters in modern sow genetics, extensive crowding in the uterine environment during gestation has become a great challenge for pig producers [1]. Piglets exposed to intra-uterine growth restriction (IUGR) during the fetal stage exhibit lower energy reserves due to their small glycogen depots at birth relative to their larger littermates [2]. This in combination with the observation that IUGR

piglets have a deviant weight-to-surface ratio leading to excessive heat loss, likely increasing the risk of hypothermia leading to higher mortality rates during the first days after birth [3]. Previous studies have suggested a strong link between colostrum intake and survival rate and as well as growth until weaning [4-6]. Furthermore, given the very low level of blood glucose levels in low compared with normal birth weight piglets, suggests that the former could benefit from an additional dose of glucose around birth [7]. However, to lower litter mortality rates effective dietary measures need to be applied at a very early stage of a piglet's life as most deaths occur three days after birth [8]. However, the exact effect on energy expenditure and nutrient oxidation has not been investigated. To assess the effect on energy expenditure of dietary interventions with a certain degree of accuracy, only a few methods are available and have been validated. One method to determine energy expenditure in pigs is by measuring heat increment (HI) based on calculations of oxygen (O₂), carbon dioxide (CO₂) and methane (CH₄) emissions detected in respiration chambers [9,10]. Furthermore, the emission volume ratio between CO₂ and O₂ termed the respiratory quotient (RQ) is regarded as an indicator of which nutrient is the primary source for oxidation in an organism. On average/in general, an RQ of 1.00, 0.81 and 0.71 corresponds to the oxidation of carbohydrates, protein and fat, respectively [9-12]. Respiratory quotients lower than 0.71 are rarely observed and can associate with the ingestion of ketogenic diets or in fasting animals [13]. To date assessing energy expenditure in IUGR piglets exposed to early dietary interventions using respiration chambers has to the best of the author's knowledge not been investigated. The study objective was to evaluate the feasibility of using respiration chambers when determining energy expenditure in combination with investigating the possible effect of two different dietary interventions (DI) on IUGR piglet metabolism. It was hypothesized that very early dietary intervention with respectively an injection of glucose (GLUC) or sow colostrum (COL) would counteract heat loss and elicit a change in nutrient oxidation resembling the metabolism of normal birth weight (Norm) piglets.

2. MATERIAL AND METHODS

2.1 Ethical approval

The experiment was conducted with approval from the Danish Experimentation Inspectorate, j.nr. 2016-15-0201-00894.

2.2 Animals and Design

A total of 44 newborn mixed female and male piglets from a commercial farm (Danish Landrace x Danish Yorkshire x Duroc, DanBred, Herlev, Denmark) were included in the study. The piglets were selected on the day of birth based on their headshape and identified either as IUGR (n = 34) or normal (n = 10) according to the categorisation described by Amdi et al., 2013 [2]. The IUGR piglets were assigned to one of three dietary treatments; Glucose (GLUC; Glucose Baxter Viaflo, Denmark) (50 mg/mL) injected subcutaneously (4 × 1.5 mL, two in the groin area and two in the neck), a tube-fed dosage of porcine colostrum (COL; 20 mL warmed to 35°C) or a placebo treatment (P; 20 mL of water). The normal birth weight piglets were given the placebo treatment (20 mL of water). Piglets from all four groups were further divided into two groups representing different levels of temperature; 20°C and 30°C. Piglets arrived within a few hours after birth at the experimental facilities. Blood glucose, rectal temperature and body weight were measured immediately upon arrival. Blood glucose was measured using a glucose monitor (Accu-Chek Aviva Nano; Roche, Basel, Switzerland), by obtaining a drop of blood from the ear by

puncturing with a disposable 21-gauge needle and rectal temperatures were recorded with a thermometer (Apotekets digital thermometer, Hørsholm, Denmark). The piglets were placed in one of two respiration chambers and after one hour, the chambers were briefly opened, the piglets removed, the chamber doors closed, and the dietary treatments were given to the piglets and the chamber doors once again opened and the piglets put back inside the chamber where they remained for another 3h. The detailed function of the respiration chambers used is described in Chwalibog et al., (2004) [10].

2.3 Sampling

The piglets were weighed again and blood was sampled via jugular vein puncture using a 22-gauge needle and Vacutainer tubes containing heparin (BD Vacutainer, Franklin Lakes, NJ, USA). Blood was then centrifuged at room temperature for 15 min at 1.20×g (CM-6MT; Elmi, Riga, Latvia), and the resulting plasma was transferred to Eppendorf tubes (Sarstedt, Nümbrecht, Germany) and immediately frozen at –20°C. The piglets were then anaesthetized with an i.m. injection of a Zoletil mix (Zoletil 50; Virbac, Kolding, Denmark) containing xylacin (Narcoxyl 20 mg/mL; MSD Animal Health, Ballerup, Denmark), ketamine (Ketaminol 100 mg/ml; MSD Animal Health) and butorphanol (Torbugesic 10 mg/ml; ScanVet, Fredensborg, Denmark), and were left covered in a pen filled with straw to achieve deep anaesthesia. Afterwards, the piglets were euthanized with an intracardial injection of 2 to 3 ml pentobarbital (200 mg/ml). The liver, spleen, kidneys, heart, lungs, adrenal glands and brain were harvested and blotted dry before these were weighed on a precision scale (Radweg, Radom, Poland). Furthermore, the stomach was removed intact, rinsed out and weighed.

2.4 Respiration chambers

In the respiratory chambers, oxygen (O₂), carbon dioxide (CO₂) and methane (CH₄) emissions were measured in 1h intervals over a 4h period. Respiratory quotients (RQ) and heat increment (HI) were calculated based on O₂, CO₂, and CH₄ values. Due to occasional technical difficulties with the respiratory chambers, it was not possible to measure oxygen (O₂), carbon dioxide (CO₂) and methane (CH₄) for all piglets involved in the experiment. However, all 44 piglets are included in the glucose, rectal measurements and organ weights.

3. RESULTS AND DISCUSSION

3.1 Results

All Norm and IUGR piglets did, respectively, exhibit similar BW at the beginning of the experiment, and all piglets lost less than 60 g BW during the experimental period. Similarly, the same pattern was observed with respect to the majority of the absolute organ weights, except for the full stomach weight where IUGR-Glu of T22 and T36 displayed markedly lower weights compared with remaining IUGR piglets.

Table 1. Body weight, organ weight, rectal temperature and blood glucose concentration

	T22				T36			
	Norm-P	IUGR-P	IUGR-Glu	IUGR-Col	Norm-P	IUGR-P	IUGR-Glu	IUGR-Col
Body weight (BW), g								
Start of experiment	1546.1±244.3	685.6±73.5	728.3±73.5	697.5±67.5	1281.0±84.0	742.7±75.2	730.6±127.5	797.0±91.1
End of experiment	1509.7±225.9	680.0±73.3	715.0±40.3	683.6±60.3	1224.6±86.3	732.8±77.4	713.1±122.9	784.1±95.2
Rectal temperature, °C								
Start of experiment	36.9±1.0	34.8±1.9	36.5±0.4	35.4±1.6	38.1±0.6	37.7±0.4	36.3±1.7	37.1±0.48
End of experiment	21,5±0,3	21,7±0,2	21,4±0,2	21,6±0,4	33,0±0,5	32,0±1,4	33,0±2,0	34,2±1,3
Organ weight, g								
Liver	47.94±13.15	14.04±2.19	18.43±2.48	17.85±1.93	34.48±2.24	19.12±3.97	19.26±3.44	20.24±3.36
Small intestine	48.56±10.31	19.11±0.88	21.65±4.31	22.13±5.36	41.16±4.73	23.91±2.15	20.95±2.11	24.74±3.72
Large intestine	11.91±1.46	8.25±3.77	6.28±1.57	6.23±1.88	9.54±0.77	8.04±1.73	5.83±1.71	6.73±1.10
Brain	36.88±1.05	32.80±1.18	32.96±1.53	31.19±1.05	33.55±1.44	33.10±0.98	31.50±1.17	31.62±1.25
Heart	10.67±2.35	5.73±0.70	5.47±0.36	6.29±1.27	8.74±0.65	5.27±0.35	5.75±1.01	6.04±0.52
Stomach, full	13.53±5.07	8.10±2.37	5.25±1.23	8.68±2.37	19.40±8.03	10.41±4.25	6.41±1.19	10.11±3.62
Stomach, empty	7.58±0.95	4.28±0.49	3.80±0.15	3.89±3.78	6.88±0.44	4.39±0.24	4.15±0.54	4.60±0.23
Stomach content	5.95±4.95	3.83±2.78	1.45±1.26	4.79±3.31	12.52±7.67	6.02±4.01	2.05±1.27	5.62±3.46
Adrenal glands	0.39±0.06	0.22±0.05	0.24±0.04	0.19±0.04	0.28±0.03	0.21±0.01	0.24±0.02	0.22±0.04
Kidney	11.75±2.17	6.52±1.97	6.36±1.53	5.14±0.56	9.73±1.22	5.83±0.73	6.42±1.33	6.48±0.591
Lungs	23.91±4.18	13.03±1.51	14.16±3.87	14.55±3.98	22.09±5.46	16.13±5.56	12.39±3.11	14.91±3.19
Spleen	1.44±0.28	0.64±0.15	0.78±0.11	0.83±0.18	1.39±0.14	0.87±0.12	0.78±0.28	1.04±0.11
Organ-to-BW, %								
Liver	3.12±0.41	2.06±0.22	2.57±0.29	2.65±0.46	2.82±0.04	2.58±0.29	2.71±0.23	2.58±0.29
Small intestine	3.23±0.60	2.83±0.21	3.07±0.77	3.21±0.57	3.35±0.22	3.27±0.14	2.98±0.26	3.15±0.26
Large intestine	0.80±0.07	1.17±0.44	0.89±0.26	0.91±0.24	0.78±0.04	1.14±0.38	0.80±0.13	0.86±0.06
Brain	2.48±0.29	4.86±0.36	4.62±0.18	4.59±0.36	2.75±0.22	4.56±0.38	4.57±0.89	4.08±0.40
Heart	0.70±0.08	0.84±0.06	0.77±0.07	0.91±0.14	0.71±0.05	0.72±0.04	0.81±0.07	0.77±0.05
Stomach, full	0.93±0.43	1.24±0.48	0.74±0.20	1.24±0.46	1.57±0.61	1.50±0.79	0.69±0.38	0.92±0.61
Stomach, empty	0.51±0.04	0.63±0.06	0.53±0.02	0.57±0.03	0.56±0.02	0.61±0.10	0.59±0.05	0.60±0.11
Adrenal glands	0.026±0.002	0.031±0.007	0.033±0.005	0.028±0.004	0.023±0.003	0.030±0.004	0.029±0.015	0.028±0.004
Kidney	0.78±0.06	0.95±0.24	0.90±0.25	0.75±0.02	0.80±0.12	0.79±0.02	0.91±0.18	0.84±0.15
Lungs	1.60±0.31	1.93±0.27	1.99±0.60	2.18±0.73	1.83±0.55	2.14±0.59	1.74±0.36	1.91±0.34
Spleen	0.095±0.008	0.094±0.016	0.109±0.014	0.121±0.023	0.114±0.010	0.118±0.004	0.108±0.034	0.136±0.029

Not until between the 1h to 2h after DI in T36 IUGR-Glu and -Col piglets displayed numerically greater HI/kg BW compared with IUGR-P (12.7% and 11.3% vs. 7.5%), whereas in T22 IUGR-Col and -P displayed similar increases which were numerically greater than IUGR-Glu piglets (13.0% and 12.8% vs. 9.1%). However, 2h and 3h after DI, respectively, only IUGR-Col and Norm-P in T22 and IUGR-Glu in T36 displayed HI/kg BW (0.56, 2.04 and 0.81%).

Table 2. Heat increment

Time	T22				T36			
	Norm-P	IUGR-P	IUGR-Glu	IUGR-Col	Norm-P	IUGR-P	IUGR-Glu	IUGR-Col
1-2h	-10.23±5.54	-14.68±2.4	-14.79±2.8	-10.46±0.87	-11.32±1.31	-13.56±3.11	-11.98±5.17	-11.47±4.41
2-3h	12.27±4.24	12.77±1.5	9.07±4.44	12.97±2.14	10.23±0.86	7.53±3.72	12.69±2.56	11.27±0.87
3-4h	-4.91±4.91	0.81±3.33	-3.12±1.97	-5.21±3.45	-1.77±1.80	-3.50±0.96	-0.34±0.36	-3.27±2.84

In the period from the 1h to 2h after DI in T36 RQ changed from 0.34-0.57, 0.28-0.55, 0.32-0.58, 0.52-0.71 for IUGR-P, -Glu, -Col and Norm-P piglets, respectively. In conclusion, only at 36°C and between 1h and 2h after DI IUGR piglets responded positively to Glu and Col treatments compared with P, while RQ values were markedly numerically lower in IUGR than Norm piglets.

Table 3. Respiratory quotients

Time	T22				T36			
	Norm-P	IUGR-P	IUGR-Glu	IUGR-Col	Norm-P	IUGR-P	IUGR-Glu	IUGR-Col
1h	0.69±0.26	0.60±0.05	0.69±0.14	0.69±0.12	0.69±0.07	0.57±0.05	0.52±0.07	0.53±0.11
2h	0.83±0.10	0.48±0.08	0.61±0.07	0.72±0.10	0.52±0.08	0.34±0.13	0.28±0.19	0.32±0.15
3h	0.93±0.10	0.68±0.08	0.77±0.10	0.78±0.11	0.71±0.03	0.57±0.06	0.55±0.02	0.58±0.11
4h	0.79±0.15	0.67±0.03	0.71±0.10	0.69±0.08	0.74±0.06	0.49±0.10	0.64±0.14	0.55±0.07
Avg.1-4h	0.83±0.08	0.63±0.02	0.71±0.10	0.80±0.03	0.69±0.05	0.54±0.00	0.56±0.03	0.52±0.09

3.2 Discussion

Because of the extensive selection for larger litter in the modern sow genetics, an increasing number of piglets within the litter have been exposed to intrauterine growth restriction due to the limited capacity of the uterus[14-16]. Consequently, these IUGR piglets have had limited supply of energy and nutrients, which have resulted in compromised energy reserves most notably their glycogen depots. Glucose as degraded from the glycogen depots is the first and primary source of energy in the immediate post-natal period. Pigs do unlike many other mammal species only possess white and not brown fat, and therefore solely depend on shivering thermogenesis to maintain body temperature. When piglets are born they are immediately exposed to a decrease in the surrounding temperature, as the temperature in the farrowing crate between 18-23 °C is substantially lower than the temperature found in the sow's utero being around 38-40 °C[17]. In addition, compared with the larger litter mates, IUGR piglets display a greater weight-to-surface ratio, which combined makes them extremely vulnerable to hypothermia and at risk of starvation because of their decreased ability to seek the sow and suckling milk[3]. Therefore, to elevate the energy levels of IUGR piglets in the immediate post-natal period, it is likely that a manual dietary intervention is necessary. As sow colostrum is the very first source of nutrition after birth, it is only natural to assume that direct oral supplementation could ameliorate the poor energy status of IUGR piglets. However, being unambiguous that colostrum is vital for piglet survival it is relative to sow's milk low in fat. A unique feature of the fatty acid composition in sow's milk is that compared with other mammals the content of C16:1n-7 is relatively high[18]. This particular fatty acid has been proposed to play a significant role in thermoregulation by activating the

mitochondrial uncoupling protein 1, which is believed to possess brown tissue-like properties[19]. Thus, from a health perspective, colostrum is naturally essential as it relative to mature milk contains great amounts of IgG, IgA and IgM, all of crucial importance for sufficient development of the immune system. However, with regards to sheer survival, the fat content in transient milk might rank higher, serving as the key component for thermoregulation in pigs and therefore a prerequisite to avoid hypothermia. Similarly, with glucose being a fast absorbable monosaccharide, it is widely considered a source of rapidly available energy. Similarly, although not to the same extent the concentration of lactose increases from colostrum to transient and mature milk.

Examining the more technical and methodological aspects of this experiment, several issues raise some concerns. Most questionable were the RQ values, which for some individuals were below the limits for physiological situations considered within the normal range. As suggested by Schutz and Ravussin (1980) [13] a number of metabolic, ventilatory as well as methodological factors could explain RQ-values below 0.7. First, considering the metabolic factors, a number of pathways lead to RQ-values below 0.7. Most notable are the respective intermediate pathways of gluconeogenesis from amino acids leading to an RQ-value of 0.4, whereas the formation of ketone bodies from fat oxidation results in an RQ-value of 0. In this context, it is noteworthy that if glucose and ketone bodies were subsequently oxidized to CO₂ and H₂O, RQ-values for the overall reactions would be similar to that of complete protein and fat oxidation, respectively. In this study, although speculative, some of these pathways could be the main route of oxidation in particularly IUGR piglets, due to their inherent deviating physiological and metabolic status at birth[20]. It is plausible that the combination of being born with low energy reserves and being prevented from ingesting sufficient amounts of colostrum immediately after birth, in this case also due to the experimental conditions, likely creates a state of starvation. In this case, one way the pig can adapt to starvation is through ketogenesis, where fatty acids[21] and ketogenic amino acids[22,23] are broken down to supply selected organs with energy. However, to directly quantify whether one or the other energy metabolism pathway correlates to a certain RQ-value would require catheterized animals. Surgical intervention is considered suitable and has been applied for larger animals [24], and thus can doubtfully be performed on IUGR piglets while simultaneously kept in a respiration chamber. Although it is relatively simple to analyze the expression of metabolic pathways in relevant tissues, the approach is limited by only having a one-time point at the end of the experiment when piglets are euthanized. Thus, longitudinal analysis of shifts in metabolic pathways correlating to shifts in HI and RQ are likely not within the range of current experimental methodologies.

These preliminary findings underline the potential differences in energy expenditure between IUGR and normal piglets and the need for further investigating the interactions between early DI and housing conditions and its effect on energy metabolism in IUGR piglets.

4. CONCLUSION

It was concluded that estimating HI and RQ of IUGR piglets using respiration chambers is associated with great technical difficulties and great uncertainties with respect to collecting reliable data. However, positive differences in HI were detected when the piglets were given the treatment after 1h. Developing and documenting the effects of suitable dietary interventions for IUGR piglets in the immediate post-natal period is still of great importance in the pursuit of lowering piglet mortality rates.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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