Effects of Late-Gestation Metabolic Energy Level on Plasma Reproductive Hormone Concentrations, Colostrum Yield, and Milk Composition in Cashmere Goats (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary metabolizable energy level during late gestation on ewe weight gain, plasma reproductive hormone concentrations, colostrum yield, colostrum composition, and lamb growth during the colostrum period. Eighteen Inner Mongolian white cashmere goats with a body weight of (39.75±2.86) kg, aged 3–4 years, and at 91 days of gestation were selected and randomly divided into 3 groups, with 6 goats per group. A basal diet was formulated according to the NRC, and the dietary metabolizable energy levels for each group were 7.70 (70% of the basal diet), 11.00 (basal diet, control group), and 14.30 MJ/kg (130% of the basal diet), respectively. The pre-trial period was from gestation day 91 to 100, and the formal trial period was from gestation day 101 to postpartum day 5. The results showed that: 1) The dietary metabolizable energy level of 14.30 MJ/kg significantly increased the average plasma estradiol (E2) concentration during late gestation, ewe body weight gain during late gestation, colostrum protein content on postpartum days 1–4, and average daily gain of lambs during the colostrum period ($P < 0.05$); dietary metabolizable energy level had no significant effect on ewe plasma progesterone (P4) and prolactin (PRL) concentrations, colostrum fat content (except on postpartum day 4), or lamb birth weight ($P > 0.05$). 2) The dietary metabolizable energy level of 7.70 MJ/kg decreased the average concentrations of plasma E2, P4, and PRL in ewes during late gestation, ewe body weight gain during late gestation, lamb birth weight, colostrum yield (except on postpartum day 5), colostrum protein content (except on postpartum days 1 and 3), and average daily gain of lambs during the colostrum period, but these effects were not significant ($P > 0.05$); it significantly decreased colostrum fat content (except on postpartum day 3). Therefore, a dietary metabolizable energy level of 14.30 MJ/kg is more suitable for ewes during late gestation, while a restricted diet with a metabolizable energy level of 7.70 MJ/kg may be used to cope with forage and feed shortages.

Full Text

Effects of Metabolic Energy Level during Late Pregnancy on Plasma Reproductive Hormone Concentrations, Colostrum Yield, and Milk Composition of Cashmere Goats

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Abstract

This study investigated the effects of dietary metabolic energy level during late pregnancy on maternal body weight gain, plasma reproductive hormone concentrations, colostrum yield, colostrum composition, and lamb growth during the colostrum period. Eighteen Inner Mongolia white cashmere goats in late pregnancy (day 91), weighing (39.75 ± 2.86) kg and aged 3–4 years, were randomly divided into three groups (n = 6). A basal diet was formulated according to NRC recommendations, with dietary metabolic energy levels of 7.70 MJ/kg (70% of basal diet), 11.00 MJ/kg (basal diet, control), and 14.30 MJ/kg (130% of basal diet). The pre-experimental period was from day 91 to 100 of pregnancy, and the experimental period was from day 101 of pregnancy to day 5 postpartum.

The results showed: (1) The 14.30 MJ/kg metabolic energy diet significantly increased the average plasma estradiol (E) concentration during late pregnancy, maternal body weight gain during late pregnancy, colostrum protein content on days 1-4 postpartum, and average daily gain of lambs during the colostrum period ($P < 0.05$). Dietary metabolic energy level had no significant effects on plasma progesterone (P) or prolactin (PRL) concentrations, colostrum fat content (except day 4 postpartum), or lamb birth weight ($P > 0.05$). (2) The 7.70 MJ/kg metabolic energy diet decreased average plasma E , P , and PRL concentrations, maternal body weight gain during late pregnancy, lamb birth weight, colostrum yield (except day 5 postpartum), colostrum protein content (except days 1 and 3 postpartum), and average daily gain of lambs during the colostrum period, but these effects were not significant ($P > 0.05$). However, it significantly decreased colostrum fat content (except day 3 postpartum).

Therefore, a dietary metabolic energy level of 14.30 MJ/kg is more suitable for ewes in late pregnancy, while restricted feeding with a 7.70 MJ/kg metabolic energy diet can be used to cope with forage shortages.

Keywords: metabolic energy; late pregnancy; reproductive hormone; colostrum

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Introduction

Inner Mongolia has an arid and semi-arid temperate continental climate with cold, windy winters. Frequent "white disasters" (severe snowstorms) limit forage supply, coinciding with the late pregnancy period of ewes. This often leads to malnutrition, weight loss, weak lambs, or even abortion during gestation. Research indicates that "white disasters" are a major factor affecting animal husbandry in Inner Mongolia. Different nutritional intake levels inevitably affect production performance. When nutrition is restricted, the maternal body mobilizes its own reserves to ensure fetal development according to the degree of restriction, but this may result in weak postpartum ewes with low milk production, hindering lamb health and growth. Therefore, studying the effects of dietary metabolic energy level during late pregnancy on ewes and lambs can provide a theoretical basis for disaster prevention and livestock protection.

This experiment investigated the effects of high and low metabolic energy diets on body weight, plasma concentrations of estradiol (E), progesterone (P), and prolactin (PRL), colostrum yield, colostrum composition, and lamb growth during the colostrum period in late pregnancy. The aim was to explore the production-enhancing effects of high-energy diets during late pregnancy and identify appropriate restriction levels for coping with forage shortages, thereby providing reasonable recommendations for feeding Inner Mongolia white cashmere goats during late pregnancy in production practice.

1.1 Experimental Diet Design

A basal diet was formulated according to NRC nutritional requirements for a 35 kg ewe with a daily gain of 51 g, expected to produce a single lamb with a birth weight of 3.1 kg. Two experimental diets were prepared at 130% and 70% of the basal diet' s metabolic energy level. The diet composition and nutrient levels are shown in Table 1 .

Table 1 Composition and nutrient levels of experimental diets (air-dry basis)

Item	Control Group	Test Group 1	Test Group 2
Ingredients			
Corn			
DDGS			
Soybean meal			
Cottonseed meal			

Item	Control Group	Test Group 1	Test Group 2
Premix			
Limestone			
NaCl			
Green hay			
Alfalfa hay			
Total			
Nutrient levels			
Metabolic energy (MJ/kg)	11.00	14.30	7.70
Crude protein			
Total phosphorus			
Neutral detergent fiber			
Acid detergent fiber			
Dry matter			

Note: Metabolic energy was a calculated value, while the others were measured values.

1.2 Experimental Animals and Grouping

Pregnancy timing was determined based on mating period and B-ultrasound examination and recorded as the basis for selecting experimental animals. Before the experiment, the original pen was cleaned and disinfected with NaOH solution. Eighteen Inner Mongolia white cashmere goats aged 3–4 years, weighing (39.75 ± 2.86) kg and at approximately day 91 of pregnancy, were randomly divided into three groups (n = 6). The control group was fed the basal diet with 11.00 MJ/kg metabolic energy; Test Group 1 was fed the experimental diet at 130% of basal metabolic energy (14.30 MJ/kg); and Test Group 2 was fed the experimental diet at 70% of basal metabolic energy (7.70 MJ/kg).

Ewes were housed individually. Before the experiment, the feeding method was barn feeding with a diet composed of mixed forage plus corn grain supplementation. Starting at day 91 of pregnancy, experimental diets were fed for a 10-day adaptation period. On day 11 at 07:00, the formal experimental period began after fasting and weighing. During the formal period, ewes were fed three times daily (07:00, 12:00, and 17:00). Table 2 shows the feeding amount per ewe during the experimental period. Ewes had free access to water, and pens were disinfected regularly. The experimental period was from December 2015 to March 2, 2016.

Table 2 Feeding amount of goats in each group

Item	Control Group	Test Group 1	Test Group 2
Feeding amount			

1.3 Measurement Methods

1.3.1 Maternal Reproductive Hormone Determination On days 1 (pregnancy day 101), 11 (day 111), 21 (day 121), 31 (day 131), and 41 (day 141) of the formal period, 5 mL of blood was collected from the jugular vein in the early morning after fasting using sodium citrate anticoagulation tubes. After collection, blood samples were centrifuged at 3,000 r/min for 5 minutes. Plasma was aliquoted into sterile centrifuge tubes and stored at -40°C for later analysis. Plasma E , P , and PRL concentrations were determined by radioimmunoassay using 200 L per sample. Labeled antigens were ^{12} I-E , ^{12} I-P , and ^{12} I-PRL, respectively. Assay kits were provided by Beijing Huaying Biological Technology Research Institute.

1.3.2 Body Weight Measurement of Ewes and Lambs Ewes were weighed after fasting on day 1 of the formal period, before delivery, and after delivery. Lamb birth weight was recorded at birth, and lamb body weight was recorded at 5 days of age.

1.3.3 Colostrum Yield and Composition Determination From days 1 to 5 postpartum, ewes were separated from their lambs. Lambs were nursed three times daily (08:00, 14:00, and 20:00). Lamb body weight was measured before and after each nursing. After each nursing, residual milk was manually expressed and collected by trained personnel. Colostrum yield was calculated as the sum of the weight difference before and after nursing plus the manually expressed milk volume. Milk samples (8 mL each) were collected at 08:00, 14:00, and 20:00 daily and mixed to form a composite daily sample. Milk protein content was determined by the Kjeldahl method, and milk fat content was measured according to GB54133-2010.

1.4 Statistical Analysis

Experimental data were initially processed using Excel 2003 and analyzed by one-way ANOVA using SAS 9.0. Duncan' s multiple comparison test was used for post-hoc analysis. Results are expressed as "mean ± standard deviation."

Results

2.1 Effects of Dietary Metabolic Energy Level on Plasma Reproductive Hormone Concentrations in Ewes

As shown in Table 3 , on day 1, plasma E concentration in Test Group 1 was slightly lower than in the control group ($P > 0.05$), but higher thereafter, with a significant difference on day 21 ($P < 0.05$). Test Group 1 had higher plasma E concentrations than Test Group 2 at all time points, with a significant difference on day 21 ($P < 0.05$). Throughout the experimental period, the average plasma

E concentration in Test Group 1 was significantly higher than in the control and Test Group 2 ($P < 0.05$), while Test Group 2 was lower than the control but not significantly ($P > 0.05$). Over time, plasma E concentrations in the control and Test Group 2 showed an increasing trend during the last 20 days of pregnancy, whereas Test Group 1 showed an overall increasing trend throughout the experimental period.

Table 3 Effects of dietary metabolic energy level on plasma E concentration of ewes (pg/mL)

Item	Day 1	Day 11	Day 21	Day 31	Day 41	Average
Control group	15.79 ± 2.82	12.08 ± 1.71	14.43 ± 2.98	17.37 ± 3.28	21.82 ± 10.48	14.31 ± 2.36
Test group 1	15.41 ± 2.46	11.90 ± 0.26	16.39 ± 3.06	18.12 ± 3.15	17.00 ± 4.57	17.75 ± 2.48
Test group 2	12.51 ± 7.16	12.38 ± 4.46	11.48 ± 4.76	15.11 ± 1.12	16.18 ± 0.13	13.53 ± 2.00

In the same row, values with the same letter or no letter superscripts mean no significant difference ($P > 0.05$), while different letters indicate significant difference ($P < 0.05$). The same applies to Tables 4, 5, 6, and 8.

As shown in Table 4 , there were no significant differences in plasma P concentrations among groups at any time point ($P > 0.05$). Test Group 1 was similar to the control group, while Test Group 2 was slightly lower. Throughout the experimental period, the average plasma P concentration in Test Group 1 was similar to the control, while Test Group 2 was slightly lower, with no significant differences among groups ($P > 0.05$). Over time, plasma P concentrations showed a slow decreasing trend.

Comparison of data in Tables 3 and 4 revealed that as pregnancy progressed, plasma P concentrations generally decreased while plasma E concentrations increased, resulting in a decreasing E /P ratio that was lowest in Test Group 1. Correlation analysis showed that plasma E and P concentrations were negatively correlated, with correlation coefficients (r^2) of -0.23, -0.79, and -0.85 for the control, Test Group 1, and Test Group 2, respectively, indicating strong negative correlations in Test Groups 1 and 2.

Table 4 Effects of dietary metabolic energy level on plasma P concentration of ewes (ng/mL)

Item	Day 1	Day 11	Day 21	Day 31	Day 41	Average
Control group	0.38 ± 0.20	0.37 ± 0.18	0.25 ± 0.14	0.24 ± 0.11	0.23 ± 0.05	0.29 ± 0.07
Test group 1	0.39 ± 0.26	0.36 ± 0.18	0.29 ± 0.03	0.25 ± 0.16	0.23 ± 0.04	0.30 ± 0.07
Test group 2	0.30 ± 0.03	0.28 ± 0.22	0.27 ± 0.18	0.25 ± 0.04	0.19 ± 0.08	0.26 ± 0.04

As shown in Table 5 , plasma PRL concentrations in all three groups showed an overall trend of decreasing first and then increasing over time. On day 21, Test Group 1 had lower plasma PRL concentrations than the control and Test Group 2, but thereafter increased more rapidly. On day 11, Test Group 2 reached its lowest value, then followed a similar trend to the control group but at lower concentrations. Throughout the experimental period, the average plasma PRL concentration in Test Group 1 was higher than in the control and Test Group 2, while Test Group 2 was lower than the control, with no significant differences among groups ($P > 0.05$).

Table 5 Effects of dietary metabolic energy level on plasma PRL concentration of ewes (IU/mL)

Item	Day 1	Day 11	Day 21	Day 31	Day 41	Average
Control group	182.46 ± 34.43	182.69 ± 27.06	166.95 ± 59.34	179.86 ± 48.64	244.00 ± 16.42	191.19 ± 30.22
Test group 1	188.12 ± 35.96	198.43 ± 27.84	159.57 ± 30.92	210.94 ± 39.49	263.69 ± 10.08	204.15 ± 38.30
Test group 2	181.80 ± 27.37	132.50 ± 45.05	169.14 ± 33.33	170.27 ± 11.69	238.59 ± 50.45	178.46 ± 38.38

2.2 Effects of Dietary Metabolic Energy Level on Body Weight Gain and Lambing Status of Ewes

As shown in Table 6 , body weight gain during late pregnancy was intermediate in the control group, not significantly different from the lowest Test Group 2 ($P > 0.05$) but significantly lower than Test Group 1 ($P < 0.05$). All groups had the same number of lambs born, with 100% survival. Comparing initial and postpartum body weights revealed that ewes in the control and Test Group 2 lost body weight (1.46 kg and 1.67 kg, respectively), while ewes in Test Group 1 gained 1.35 kg.

Table 6 Effects of dietary metabolic energy level on body weight gain and lambing status of ewes

Item	Control Group	Test Group 1	Test Group 2
Ewes' body weight (kg)			
Initial	38.72 ± 1.24	41.20 ± 1.52	40.03 ± 1.57
Before delivery	41.91 ± 2.87	47.83 ± 2.24	42.19 ± 3.14
After delivery	37.26 ± 2.20	42.55 ± 4.15	38.36 ± 3.10
Body weight gain during late pregnancy (kg)	3.19 ± 0.71	6.63 ± 0.89	2.16 ± 0.65
Number of lambs			
Survival rate of lambs (%)	100	100	100

2.3 Effects of Dietary Metabolic Energy Level on Colostrum Yield and Composition

As shown in Table 7 , colostrum yield at each time point ranked from highest to lowest as Test Group 1, control, and Test Group 2. There were no significant differences among the three groups on days 1 and 4 ($P > 0.05$). On day 2, Test Group 1 was significantly higher than the control and Test Group 2 ($P < 0.05$), while the control and Test Group 2 did not differ significantly ($P > 0.05$). On day 3, Test Groups 1 and 2 did not differ significantly from the control ($P > 0.05$), but Test Group 1 was significantly higher than Test Group 2 ($P < 0.05$). On day 5, all groups differed significantly ($P < 0.05$).

Colostrum fat content showed a "decrease-increase-decrease" trend postpartum. The control and Test Group 1 were significantly higher than Test Group 2 on days 1 and 2 ($P < 0.05$). On day 3, Test Group 1 was higher than the control ($P > 0.05$) and significantly higher than Test Group 2 ($P < 0.05$). On day 4, the ranking was Test Group 1 > control > Test Group 2, with highly significant differences among groups ($P < 0.01$). On day 5, Test Group 1 and the control were significantly higher than Test Group 2 ($P < 0.05$).

Colostrum protein content decreased over time, rapidly on days 1-2 and more slowly thereafter. From days 1-4 postpartum, Test Group 1 had highly significantly higher colostrum protein content than the control and Test Group 2 ($P < 0.01$). On day 5, Test Group 1 was significantly higher than Test Group 2 ($P < 0.01$). The control group was significantly higher than Test Group 2 on days 1 and 3 ($P < 0.01$).

Table 7 Effects of dietary metabolic energy level on colostrum yield and milk composition

Item	Group	Day 1	Day 2	Day 3	Day 4	Day 5
Colostrum yield (kg/d)	Control	0.65 ± 0.09	0.50 ± 0.05	0.79 ± 0.07	0.73 ± 0.04	0.82 ± 0.06
	Test 1	0.94 ± 0.10	0.66 ± 0.06	0.67 ± 0.15	0.53 ± 0.05	0.65 ± 0.06
	Test 2	0.31 ± 0.18	0.41 ± 0.11	0.27 ± 0.24	0.64 ± 0.03	0.94 ± 0.10
Milk fat content (%)	Control	5.87 ± 0.35	6.46 ± 0.49	5.47 ± 0.41	5.43 ± 0.47	5.53 ± 0.31
	Test 1	4.97 ± 0.37	4.92 ± 0.28	4.18 ± 0.39	5.82 ± 0.58	6.25 ± 0.35
	Test 2	4.65 ± 0.27	3.52 ± 0.34	3.91 ± 0.36	3.95 ± 0.48	3.32 ± 0.40
Milk protein content (%)	Control	15.66 ± 0.83	17.35 ± 0.95	10.84 ± 0.59	4.94 ± 0.51	3.58 ± 0.37
	Test 1	12.76 ± 0.64	5.96 ± 0.35	7.59 ± 0.37	7.59 ± 0.37	5.46 ± 0.48
	Test 2	3.97 ± 0.39	3.92 ± 0.39	3.95 ± 0.48	3.32 ± 0.40	2.98 ± 0.41

In the same column, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while different letters indicate significant difference ($P < 0.05$).

2.4 Effects of Ewes' Dietary Metabolic Energy Level on Lamb Body Weight during Colostrum Period

As shown in Table 8 , lamb birth weight in Test Group 1 was higher than the control ($P > 0.05$) and significantly higher than Test Group 2 ($P < 0.05$), while the control was higher than Test Group 2 ($P > 0.05$). Average daily gain of lambs during the colostrum period in Test Group 1 was significantly higher than in the control and Test Group 2 ($P < 0.05$). Test Group 2 was lower than the control but not significantly different ($P > 0.05$).

Table 8 Effects of ewes' dietary metabolic energy level on lambs' body weight during colostrum period

Item	Control Group	Test Group 1	Test Group 2
Birth weight of lambs (kg)	2.93 ± 0.31	3.18 ± 0.22	2.58 ± 0.33
Lambs' ADG during colostrum period (g/d)	117.33 ± 27.39	159.36 ± 5.70	115.60 ± 21.81

Discussion

3.1.1 Plasma E Concentration

Estrogen secreted by the placenta promotes uterine muscle thickening and mammary gland development while producing prostaglandins to increase blood flow between the uterus and placenta, thereby promoting fetal growth. The higher plasma E concentration in Test Group 1 ewes during late pregnancy was more favorable for pregnancy maintenance and fetal development. The changing trend of plasma E concentration was consistent with results from studies on Jianchang black goats by Xu Rui and dairy cows by Sun Xiaojing.

3.1.2 Plasma P Concentration

P is a steroid hormone secreted mainly by luteal cells, with small amounts from follicular theca cells and placenta during pregnancy. In non-primates, the withdrawal of progesterone triggers parturition in late pregnancy. In goats and other animals, pregnancy maintenance throughout gestation depends primarily on P secreted by the corpus luteum. In this study, Test Group 1 had higher plasma P concentrations during late pregnancy, which helped maintain uterine quiescence, indicating that higher dietary metabolic energy contributes to pregnancy maintenance. Plasma P concentrations showed a decreasing trend from day 91 of pregnancy, consistent with studies on Jianchang black goats by Xu Rui and dairy cows by Yao Lulian, but slightly different from results on Qinghai semi-fine wool sheep by Wang Ying' an et al., possibly due to breed differences or because the blood sampling interval was longer than the regulatory effect of the neural center on hormone secretion. Future studies should shorten the sampling interval appropriately without causing stress to obtain more detailed data.

3.1.3 Plasma PRL Concentration

During pregnancy, PRL works with E and P to maintain mammary alveolar system development, though P inhibits PRL action during gestation. The results showed that plasma PRL concentrations in all three groups increased during late pregnancy, consistent with studies on dairy cows by Yao Lulian and Liaoning cashmere goats by Zhang Xiaoying et al., with Test Group 1 having the highest levels throughout late pregnancy. Within the design range of this experiment, no significant differences in plasma PRL concentration were observed, though the high metabolic energy Test Group 1 had higher concentrations than the other two groups.

3.1.4 Comprehensive Effects

The "hypothalamic-pituitary-gonadal" axis is the main humoral regulatory pathway for E and P, while prolactin-releasing factor and prolactin-inhibiting factor are the primary humoral regulators of PRL. Humoral regulation mainly occurs through negative feedback. Small doses of PRL promote E and P synthesis, while large doses inhibit their synthesis and secretion through negative feedback. However, the different trends in plasma E and P concentrations in this study may be because plasma PRL concentration was at a level that promoted E but inhibited P. Plasma PRL concentration may have increased under neural regulation during the experimental period, but its lactogenic effect was inhibited by P, primarily working with E and P to promote mammary alveolar development in preparation for lactation. Detailed mechanisms of dietary metabolic energy effects on reproductive hormones require further study of physiological changes in the hypothalamus and adenohypophysis, as well as expression of related genes such as kiss-1, G protein-coupled receptor 54 (GPR54), and type 2 deiodinase gene (Dio2).

In summary, moderately increasing dietary metabolic energy level is beneficial for ewes in late pregnancy, while restriction at 70% of metabolic energy requirements has minimal impact.

3.2 Effects of Different Dietary Metabolic Energy Levels during Late Pregnancy on Colostrum Composition Changes

Colostrum is milk produced by the mammary gland during parturition or the first 3-5 days postpartum and is crucial for ensuring the healthy growth of newborn animals. According to Wang Xiang et al., dairy cow colostrum composition changes substantially during days 1-5 postpartum, approaching normal milk composition after day 5. Ma Yanfen et al. reported that bovine colostrum fat content gradually decreased within 48 h postpartum, increased from 48 h to day 5, then decreased again after day 5. Our results were similar: colostrum fat content decreased on days 1-2, increased on days 2-4, and decreased after day 4. Colostrum protein content changed dramatically, especially on days 1-2 postpartum, because colostrum protein is mainly immunoglobulins whose concentration decreases within 24 h postpartum. Liu Chang studied the composition and main nutrient changes in Laoshan dairy goat milk during different lactation periods, finding that all main nutrients except lactose showed the highest concentrations on day 1 postpartum, consistent with our results.

3.3 Effects of Different Dietary Metabolic Energy Levels during Late Pregnancy on Ewe Body Weight and Lamb Weight Gain

Fetal growth is rapid during late pregnancy, requiring high energy intake. High metabolic energy diets not only meet fetal growth needs but also increase maternal body weight, which helps mitigate reduced colostrum yield and body condition loss caused by poor postpartum appetite, low feed intake, and decreased digestive function. High metabolic energy diets during late pregnancy and postpartum increased colostrum protein and fat content, promoting lamb growth during the colostrum period. Further research is needed on the effects of colostrum from ewes fed different metabolic energy diets on lamb immune function.

Conclusion

Considering Inner Mongolia' s natural environment and forage resources, we recommend providing ewes in late pregnancy with diets containing 14.30 MJ/kg or higher metabolic energy during winter when forage supply is adequate to ensure pregnancy needs and promote lamb growth during the colostrum period. During "white disaster" periods or other times of nutritional scarcity, diets with 7.70 MJ/kg metabolic energy can maintain pregnancy without causing significant adverse effects on lamb growth during the colostrum period, thereby coping with disasters and reducing losses.

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Note: Figure translations are in progress. See original paper for figures.