

Effects of Feed Restriction and Exercise on Glucose Tolerance in Sheep

(Pengaruh Pembatasan Pakan dan *Exercise* terhadap Kadar Glukosa pada Domba)

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Abstrak

Penelitian ini bertujuan untuk mengetahui kecepatan penggunaan glukosa dalam darah pada ternak domba yang diberi pakan terbatas dan berjalan diatas *treadmill*. Delapan belas ekor domba merino kastasi berumur 8-9 bulan dikelompokkan berdasarkan bobot badan yang selanjutnya secara random dialokasikan ke dalam tiga perlakuan. Selama Periode I (45 hari) domba dalam Perlakuan I mengalami pembatasan pakan dan jalan di atas *treadmill* selama 2,5 jam sehari. Domba dalam Perlakuan II mengalami pembatasan pakan tetapi tidak berjalan (*not-exercise*). Domba dalam Perlakuan III diberi pakan *ad libitum* dan tidak berjalan diatas *treadmill*. Selama Periode II semua ternak diberi pakan *ad libitum* dan tidak berjalan diatas *treadmill*. Analisis ragam menunjukkan bahwa *exercise* meningkatkan kecepatan penggunaan glukosa darah pada ternak yang mengalami pembatasan pakan. Namun demikian pembatasan pakan baik disertai dengan *exercise* maupun tidak menurunkan toleransi terhadap glukosa. Pemberian pakan kembali secara *ad libitum* menormalkan toleransi terhadap glukosa. Disimpulkan bahwa pembatasan pakan dapat menurunkan kecepatan penggunaan glukosa dalam darah sehingga kadar glukosa darah tidak terus mengalami penurunan dan homeostasis dipertahankan.

Kata Kunci: Domba, Pembatasan Pakan, Glukosa, Homeostasis

Introduction

The glucose tolerance test is a simple technique for assessing an animal's ability to maintain glucose homeostasis through insulin secretion. Homeostasis mechanisms involve the net outcomes of glucose influx and outflow from the blood pool.

Glucose concentrations and insulin secretion are influenced by such factors as dietary treatments. Rao (1995) found that nutritional restriction to 60% of *ad libitum* intake in weanling rats over an 8 week period decreased insulin secretion by the pancreas. Similarly, King et al. (1988) provides evidence that reduced insulin secretion in rats is responsible for lowered post exercise insulin response to glucose challenge. Fasting glucose levels are lower in underfed than in fully fed sheep (Canacoo, 1995; Bigdeli, 1996 and Rao, 1995).

Carcass fatness is linked to sheep insulin status (Munro et al., 1985), Francis et al. (1994) claimed that the progeny of rams

selected for fast or slow glucose clearance after intravenous glucose tolerance, differed significantly in glucose tolerance. Carcasses of fast line progeny have more subcutaneous fat at the same carcass weight than do carcass of slow line progeny. The former has higher insulin concentrations in response to intravenous glucose challenge. Insulin is shown to be positively related to carcass adipose tissue and negatively related to carcass protein (Trenkle and Topel, 1978). Insulin stimulates body weight gain which is primarily accounted for by an increase in fat mass. Insulin also promotes an increase in protein accumulation (Woodward and Emery, 1989).

Previous experiments (Yuwono, 2000) have demonstrated that sheep fed at sub-maintenance levels for 45 days lost live weight which was reflected in body component loss (fat, protein, water and ash). During resumption of full feeding animals that had previously experienced nutritional stress grew faster and deposited protein at a faster rate than did the control (Yuwono, 2000). Since glucose

is the preferred energy-yielding substrate for protein synthesis (Mahyudin, 1990) an increase in the rate of protein deposition might result in an increase in glucose utilisation that was reflected in faster glucose clearance from the circulation. Therefore, the purpose of this experiment was to investigate animal response to underfeeding and exercise in terms of glucose tolerance (glucose clearance).

Research Methods

Experimental Design

Eighteen merino wethers (8-9 months of age) were blocked on live weight in randomized block design for six replicates of three treatments. The treatments were then randomly allocated to animals within blocks. Treatment I (Group I) included an underfeeding regimen and exercise (walking on a treadmill at an incline of 5° at 1.1 m/s for 2.5 h/d, 6 d/week). The energy expenditure was approximately 1.3 Em. Treatment II (Group II) included the under feeding regimen only. Treatment III (Group III) was the control group, which involved full feeding and no exercise throughout the experiment. Animals in Groups I and II were underfed so that their rates of live weight loss were very similar during the allocated seven-week period (Period I). After Period I, exercise and underfeeding were terminated and all animals were fully fed for four weeks (Period II).

Experimental Procedures

(a) Feed and feeding

During the adaptation period, the wethers were kept in individual pens and fed a diet containing pelleted Lucerne hay (*Medicago sativa*). Following this period, the amounts of feed given to the animals in Groups I and II were adjusted progressively in order to ensure that animals in the two groups lost live weight at similar rates. Animals in Group III were fully fed throughout the experiment.

(b) Jugular catheterization

Each animal was catheterized in the left and right external jugular veins two days before the glucose tolerance test was conducted. Details of procedures for catheterization and maintenance of

catheter patency as described by Teleni and Annison (1986).

(c) Intravenous glucose tolerance test (IVGTT)

Animals were denied access to feed 18 - 20 h prior to and during IVGTT, but they were allowed access to drinking water that was available at all times. Intravenous Glucose Tolerance Tests were undertaken on Days 0, 17, 37 (Period I) and on Day 56 (Period II). Details of IVGTT procedures were as described by Wastney *et al.* (1982). Glucose (0.3 g/kg live weight) was injected through a jugular catheter over a 1-min period. And the catheters were then flushed with saline. Blood samples were taken via the other jugular catheter that had not been used for glucose injection. Blood samples (2.5 mL) were collected 5 and 2 min before glucose injection and at intervals of 3, 4, 5, 7, 10, 15, 20, 25, 30, 38, 45, 52 and 60 min after injection. Blood samples were stored on ice in heparins tubes and then centrifuged at 3000 g for 10 min at 4° C. Plasma was separated and stored in 5 mL vials stored at -20° C until required for analysis.

Glucose Solution

Glucose load was made up as a 50% solution (wt/v), which was prepared by dissolving D-glucose (laboratory grade; Ajax Chemicals, Auburn, NSW, Australia) in distilled water and sterilized by filtering the solution through a pre-filter (GF/D, 47 mm in diameter. Whatman International Ltd, England) and then through a second filter (GF/F, 47 mm in diameter. Whatman International Ltd, England) into sterile 20 L bottles.

Laboratory Analysis

Plasma was analyzed for glucose with a Technicon Autoanalyser II (Bran and Leubbe Pty, Ltd, Germany) using the Technicon Calorimetric method. Number 339-02 as described by Bittner and Manning (1967). This technique is based on the reaction of cupric neocuprione chelate (2,9-dimethyl-1-1-,10 phenanthroline) with glucose.

Calculations

Glucose disappearance rates were calculated using the following equation: $\text{Log } C_t = \text{Log } C_0 - k \times t$ (Sasaki *et al.*, 1984).

Using incremental plasma glucose values (mM) between 10-60 min after glucose injection, C_t is the plasma glucose increment at time t (min). C_0 is the plasma glucose increment at $t = 0$ while k is the disappearance rate of injected glucose from plasma. In addition, $t_{-1/2}$, the time required to reduce glucose concentration in the circulation to one-half, was calculated from linear regressions of log change of glucose concentration from the basal line value over a 10-60 min period as described by Westney *et al.* (1982).

Glucose clearance rate was also expressed as: $k = \ln 2/t_{-1/2} \times 100$

where k is glucose clearance rate (%/min) and $t_{-1/2}$ is time required to reduce glucose concentration in the circulation to one-half.

Statistical Analysis

Significant differences in glucose disappearance rates was tested using one-way ANOVA according to Daniel (1991), using statistical software SPSS (SPSS Inc, release 6.1, Illinois, USA). If any significant differences were apparent between treatments, a post-hoc multiple range test using Honestly Significant Difference (HSD) was then applied.

Results and Discussion

Mean values of $T_{-1/2}$ and glucose clearance in animals in Groups I, II and III during Periods I and II are presented in Table 1 and Figure 1.

Mean value of $T_{-1/2}$ of glucose steadily increased as the duration of underfeeding period progressed. Exercise involvement during underfeeding slowed down the rate of changes in $T_{-1/2}$ of glucose as the underfeeding period advanced. The mechanisms underlying such phenomenon are not clearly understood. It is possible that exercise partially overcome nutritional stress by activating glucose clearance by means of different routes which do not depend on insulin action. A similar study on mares showed that $T_{-1/2}$ of glucose following glucose challenge was slower in energy restricted mares than in non-restricted control mares (Sticker *et al.*, 1995). Similar findings have also been reported in sheep (Waghorn *et al.*, 1987), this suggesting that well-fed animals are less well-adapted to

glucose conservation than those fed below maintenance.

$T_{-1/2}$ of glucose refers partly to the functioning of insulin amount released by the pancreas in response to glucose challenge, as well as to insulin action, by means of insulin receptors (Basset, 1978). Therefore, the amount of insulin which reaches the peripheral tissues after the glucose tolerance test plays a significant role in glucose disappearance rate from the circulation. A study on pigs (Vandergrift *et al.*, 1985) found that there were lower insulin concentration and higher growth hormone (GH) secretion in response to glucose challenge in underfed than in fully-fed pigs. Growth hormone influences carbohydrates and lipid metabolism by decreasing insulin sensitivity in pigs (Dunshea *et al.*, 1992). Growth hormone elevation is likely to decrease glucose tolerance by reducing insulin-mediated glucose uptake through increasing FFA levels (Davidson, 1987). It is possible that GH induces peripheral insulin resistance independent of plasma FFA and insulin levels (Jason *et al.*, 1999).

Reduced insulin response to oral glucose intake in rats after exercise is due to reduced insulin secretion as evidenced by lower C-peptide release (King *et al.*, 1988). Insulin and C-peptide are secreted from the pancreas in equimolar amounts (Horwitz *et al.*, 1983) but only insulin is extracted by the liver (Licinio-Paixao, 1986), which means that plasma C-peptide is a good indicator of β -cell activity. Despite low insulin response to glucose challenge in exercised subjects, glucose tolerance remains normal (Seals *et al.*, 1984). In the current experiment, impaired glucose tolerance in exercised animals was observed. The discrepancies in results are probably due to having combined severe feed restrictions with exercise.

Fluckey *et al.* (1994) found that low insulin response to oral glucose after exercise was due to an increase in insulin clearance since C-peptide response was unchanged. Rao (1995) reported that insulin and glucagon secretions decreased in weanling rats which were restricted to 60% *ad libitum* intake over 8 weeks. Peripheral and portal hormone insulin levels were similar, thus indicating that hepatic extraction was not altered.

Table 1. Mean of $T_{-1/2}$ and Glucose Clearance Rate in Animal Trial (by groups) during Periods I and II

	Group	Day 0	Day 17	Day 37	Day 56
$T_{-1/2}$ (minute)	I	38.82 ± 10.14	51.09 ± 6.94	64.49 ± 7.06^{ab}	45.56 ± 7.89
	II	35.44 ± 9.66	57.60 ± 2.40	80.83 ± 5.78	51.41 ± 9.80
	III	41.68 ± 9.80	47.67 ± 7.48	45.05 ± 4.32^b	41.26 ± 8.34
	P	0.84	0.73	0.01	0.72
Clearance Rate (%/min)	I	1.77 ± 0.40	1.50 ± 0.23	1.14 ± 0.12^a	1.70 ± 0.26
	II	2.26 ± 0.63	1.21 ± 0.05	0.88 ± 0.06^a	1.66 ± 0.34
	III	1.78 ± 0.50	1.49 ± 0.25	1.79 ± 0.25^b	1.72 ± 0.28
	P	0.67	0.53	0.003	0.99

^{ab} Means with the same superscript in the same column do not differ significantly

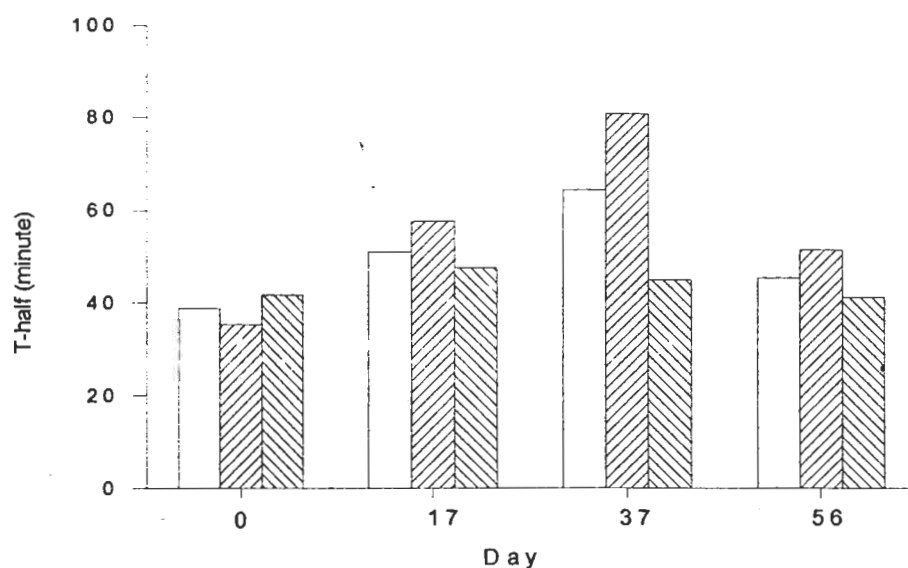


Figure 1. Mean $T_{-1/2}$ in animals in Groups I (□), II (▨) and III (▩) during Periods I (Days 0, 17 and 37) and II (Day 56)

Consequently, it can be inferred that impaired glucose clearance rates in underfed plus exercised as well as underfed but not exercised on day 37 of the underfeeding period in the present experiment are the result of insulin deficiency reaching peripheral tissues after glucose injection.

Such deficiency might be due to a low insulin secretion rate by the pancreas or to a

fast insulin clearance rate by the liver. Furthermore, changes in hormonal profile (insulin and GH) might be responsible for changes in glucose clearance rates.

It is probable that insulin deficiency results in reduced rate of recruitment glucose transporters (Glut-4) to plasma membranes of adipose tissues and striated muscle cells which then suppress glucose uptake by these tissues

and subsequently gives rise to a slower glucose clearance rate.

The finding that there was no significant difference in glucose clearance rates and $T_{-1/2}$ during resumption of full feeding amongst the groups might have been due to animals in Groups I and II having compensated for reduced insulin concentration. Bigdeli (1996) reported that, three weeks after resumption of full feeding, previously underfed sheep had lower insulin concentrations during glucose infusion. If Groups I and II in the current experiment have lower insulin concentrations in response to glucose load than did Group III and, if glucose clearance and $T_{-1/2}$ were the same, then the former groups could have been more sensitive to insulin.

The finding that resumption of full feeding showed no significant difference in glucose clearance rates and $T_{-1/2}$ between Groups I and III was in agreement with Bigdeli's observations (1996) and contradictory to that of Cole *et al.* (1993). In the latter study sheep had been deprived of feed and water for only 3 days, followed by resumption of full feeding during which glucose tolerance tests were performed.

Conclusions

Exercise reduces the rate of changes of glucose clearance over the underfeeding period. This suggests that glucose utilisation by exercised animals is faster than that of underfed animals but not exercised. It might be speculated that exercise partly overcome nutritional stress by activating glucose clearance through routes which is independent on insulin action.

Resumption of full feeding can restore normal glucose tolerance in previously underfed and exercised as well as underfed and not exercised animals.

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