

Feed Restriction Does Not Impair Insulin Sensitivity, but Exercise and Resumption of Full Feeding Increase Insulin Sensitivity and Blood Flow Across the Hind-Limb Muscles

(Pembatasan Pakan tidak Menurunkan Kepekaan terhadap Insulin tetapi *Excercise* dan Pemberian Pakan Penuh Meningkatkan Kepekaan Insulin dan Aliran Darah Otot Kaki Belakang Domba)

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ABSTRAK: Tujuan penelitian ini adalah untuk mengetahui kepekaan terhadap insulin dan pemanfaatan glukosa oleh kaki belakang domba yang mendapatkan perlakuan pembatasan pakan dan *excercise* serta pada domba yang diberi pakan penuh dan tidak *excercise*. Domba sebanyak 18 ekor berumur 8-9 bulan dikelompokkan berdasarkan bobot badan dalam rancangan acak kelompok terdiri dari tiga perlakuan dengan enam ulangan. Terdapat dua periode dalam penelitian ini, pada periode I (45 hari) domba dalam perlakuan I dibatasi pemberian pakannya dan *excercise* selama 2,5 jam perhari, 6 hari seminggu dengan kecepatan 1,1 m per detik. Domba dalam perlakuan II hanya mengalami pembatasan pakan saja, sedangkan domba dalam perlakuan III diberi pakan *ad libitum* dan tidak *excercise*. Pada periode II (30 hari), semua domba dalam perlakuan I, II dan III diberi pakan *ad libitum* dan berhenti *excercise*. Kepekaan terhadap insulin ditandai oleh ekstraksi glukosa selama hiperisulim pada kaki belakang tidak berbeda nyata ($P>0.05$) antar perlakuan pada akhir periode I. Ekstraksi glukosa (rata-rata $\pm$ salah baku) masing-masing perlakuan adalah $4,71 \pm 0,9$; $3,70 \pm 0,72$; $3,49 \pm 0,54$ %/kg otot. Pada minggu kedua periode II, kepekaan terhadap insulin cenderung lebih tinggi ($P=0.064$) dengan nilai ekstraksi glukosa (rata-rata $\pm$ salah baku) untuk perlakuan I, II dan III masing-masing adalah $3,79 \pm 0,26$; $3,88 \pm 0,39$; $2,99 \pm 0,41$ %/kg otot. Pada akhir periode I, laju aliran darah yang melalui kaki belakang untuk perlakuan I dan II masing-masing lebih rendah 19 dan 24% dibandingkan dengan perlakuan III sehingga berakibat pemanfaatan glukosa lebih rendah ($P<0,05$). Pada periode II, laju aliran darah dan pemanfaatan glukosa tidak berbeda nyata ($P>0,05$) antara ketiga perlakuan. Disimpulkan bahwa pembatasan pakan tidak menurunkan kepekaan terhadap insulin. *Excercise* dan pemberian pakan secara *ad libitum* setelah sebelumnya mengalami pembatasan pakan akan meningkatkan kepekaan terhadap insulin.

Kata Kunci: Domba, glukosa, ekstraksi, insulin, laju aliran darah, *excercise*, pembatasan pakan

Introduction

Previous experiment showed clearly that prolonged underfeeding of sheep reduces glucose clearance rate from its circulation, following an intravenous injection of a bolus of glucose (Yuwono, 2004). The glucose clearance rates return to normal values when underfed animals return to full feeding. It is uncertain whether the change in the glucose clearance rate in underfed animals is caused by reduced insulin secretory responses to glucose or impaired insulin action at tissue level. Factors such as pancreatic secretory response to glucose load, insulin clearance rate from the blood and the action of insulin on target tissues are responsible for glucose clearance (Bergman, 1989). Since skeletal muscle is a major site for glucose disposal, reduction in glucose uptake by this tissue has a major impact on glucose clearance rate. Insulin mediates glucose

uptake by muscle cell by attaching to its cell membrane receptors thus creating an interaction that initiates a cascade of events resulting in translocation of glucose transporters (Glut-4) to membranes (Lienhard *et al.*, 1992). Underfeeding can increase the number of insulin receptors thus providing more binding sites for insulin (Knott *et al.*, 1992) which result in increases in insulin sensitivity. Insulin sensitivity is also governed by the number of Glut-4 in the plasma membrane (Hansen *et al.*, 1998). Previous authors have already reported that both calorie restriction (Dean *et al.*, 1998 and Edward *et al.*, 2007) and exercise (Brozinick *et al.*, 1993 and Hansen *et al.*, 1998) can increase the number of Glut-4 at muscle cell surfaces.

It is hypothesised that underfeeding of sheep increases hind-limb muscle sensitivity to insulin and exercise during underfeeding has an additive effect on hind-limb muscle sensitivity to insulin. The aim

of the current experiment was to determine insulin sensitivity of hind-limb muscles and glucose uptake by the hind-limb muscles of sheep that had been underfed and exercised and sheep that had resumed full feeding and ceased exercising.

Research Methods

Animals and Feeding

Eighteen sheep wethers 8-9 months of age, pelleted lucerne hay (*Medicago sativa*), arterial catheters made of polyethylene 0.86 mm ID x 1.27 mm OD. Jugular venous catheters for left and right external jugular veins made of Polyethylene 1.0 mm ID x 1.5 mm OD and a leg vein catheter made of Clear vinyl tubing 0.86 mm ID x 1.27 mm OD. The Polyethylene catheters were purchased from Critchley Electrical Products Pty Ltd, Silverwater, NSW, Australia. Aseptic and sterile glucose load made up as a 50% solution (wt/v) and prepared by dissolving and filtering D-glucose was obtained from Laboratory grade; Ajax Chemicals, Auburn, NSW, Australia). A stock solution containing 100 IU/ mL beef mono component insulin was purchased from Novo Nordisk Pharmaceutical Pty Ltd, Denmark. Stock solutions of 37 MBq/mL of tritiated water (^3HOH) diluted in aliquots of 100 mL to give concentrations of 925 kBq/mL and 9.25 kBq/mL.

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 animals were fed according to the feeding regimes outlined in Table 1. The amounts of feed given to the animals in Groups I and II were adjusted progressively in order to ensure that animals in these groups lost live weight at a similar rate. Animals in Group III were fully fed throughout the experiment.

Table 1. Treatments imposed on animals in Groups I, II and III during Periods I and II

Period	Group I	Group II	Group III
I (45 days)	Underfed + exercise	Underfed only	Full feeding only
II (21 days)	Full feeding only	Full feeding only	Full feeding only

Experimental Design

Eighteen wethers were used in the experiments. The wethers were blocked on live weight in randomised block design for six replicates of

three treatments. 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 50 at 1.1 m/s for 2.5 h/d, 6 d/week (energy expenditure was approximately 1.3 Em)]. Treatment II (Group II) included the underfeeding regimen only. Treatment III (Group III) was the control group, which involved full feeding and no exercise throughout the experiment. After Period I, exercise and underfeeding were terminated and all animals were fully fed for four weeks (Period II).

All animals were weighed within the same time period (60 min) on the same day each week. Weighing was conducted before feeding. Lateral saphenous vein, femoral artery jugular vein catheterisation and hind-limb muscle preparation for the current study of muscle metabolism was similar to that described by Domanski *et al.* (1974), with some modifications according to procedures described by Teleni and Annison (1986). Hyperinsulinemic euglycemic clamp study. The study was conducted at the end of Period I and in Week 2 of Period II (resumption of full feeding). In order to produce hyper insulinaemia at control glucose concentrations, a euglycaemic glucose clamp was performed according to the methods reported by De Fronzo *et al.* (1979). Insulin was administered as a single continuous infusion through a jugular catheter at the rate of 6 mU/min per kg live weight, as described by Weekes *et al.* (1983). Blood flow was estimated using the ^3HOH technique described by Oddy *et al.* (1981).

Blood was collected over 10 minute intervals into 10 mL tubes kept in an ice bath. Plasma separation. Blood samples were centrifuged in a refrigerated (4°C) centrifuge (GAR centrifuge, Beckman Instrument, USA) at 3000 g for 10 minutes. Plasma was collected into 5 mL plastic vials and stored frozen at -20°C until required for analysis. Variables measured were blood flow across the hind limb, glucose uptake by hind-limb muscle and glucose extraction by hind-limb muscle.

Statistical Analysis

One-way ANOVA according to Daniel (1991) was applied in this study. Where there was a significant effect of treatments a post-hoc multiple range test using Honestly Significant Difference (HSD) was undertaken in order to determine the significance of difference between means.

Results and Discussion

Steady State Glucose Infusion Rate (SSGIR)

Data on SSGIR during infusion of insulin at the end of Period I and Week 2 of resumption of full feeding (Period II) are presented in Table 2. The 'glucose clamp' procedure was successful as indicated by the relative stability in arterial glucose concentration between pre-infusion and during infusion periods in Periods I and II. Under steady state conditions, glucose infusion rate during insulin infusion would be equal to the amount of glucose being translocated out of glucose space (i.e., glucose metabolised) provided that endogenous glucose production is completely suppressed (deFronzo *et al.*, 1979 and Steven *et al.*, 2005). Brockman (1983) has reported that insulin concentrations of 60 $\mu\text{U/mL}$ during euglycaemia in sheep inhibited the appearance rate of endogenous glucose by 50%. Maximal inhibition (100%) occurred during combined hyperglycaemia ($\sim 5\text{mM}$) and hyperinsulinaemia ($\sim 200\mu\text{U/mL}$). An insulin dose of 6 mU/min/kg in sheep produced arterial plasma insulin concentrations of about 630 $\mu\text{U/mL}$ according to Weekes (1985). The current experiment has not ascertained whether endogenous glucose production was completely suppressed in each group of animals. The rate of insulin infusion would suggest that complete suppression of endogenous glucose production had taken place. However, if this assumption is incorrect, the SSGIR values presented in Table 2, have underestimated the amount of glucose translocated out of glucose space.

The finding that animals undergoing prolonged underfeeding (Group II) had lower mean SSGIR values than those of the fully fed animals at the end of Period I, appears to contradict with the observation that mean values of glucose A-V concentration difference across hind-limb muscles which are expressed as per mU insulin infused per kg muscle were not different between Groups II and III. The portal drained viscera and the skeletal muscle are the two major sites of glucose disposal in sheep (Weekes, 1979). The entire splanchnic bed accounts for only 5% of disposal, whereas muscle could account for 85% of glucose disposal of intravenously infused glucose in humans (Baron *et al.*, 1988). Therefore changes in skeletal muscle glucose uptake during hyperinsulinaemia can significantly affect glucose infusion rates required to maintain reference glucose concentrations.

Because under steady state conditions the amount of exogenous glucose infusion required to maintain euglycaemia is the sum of the insulin-induced increase in glucose utilisation and the insulin-induced suppression of glucose production, it is difficult to conclude whether the low mean value of SSGIR in Group II was due to insulin resistance by muscles or to a reduction in decrement of endogenous glucose production. There would be less probability of the latter factor occurring as previously discussed in the preceding paragraph. It is possible that lower SSGIR values for Groups I and II were due to lower reference glucose concentrations for the two groups. Increasing the reference value so that it equals that of Group II might have resulted in higher SSGIR values for Groups I and II.

The fact that, at the end of Period I, there was no significant difference in SSGIR between Groups I and III although values for both these groups were higher than that for Group II, probably reflect the presence of an insulin-independent mechanism which facilitates the uptake of glucose by skeletal muscles of the exercised animals in Group I.

When full feeding was resumed for 14 days and glucose concentrations returned to normal values, previously underfed and exercised and underfed and not exercised animals had higher SSGIR values than the control group. This phenomenon might be due to adaptive responses to resumption of full feeding rather than the effect of the last bout of exercise or underfeeding. Resumption of full feeding brought about an increase in insulin-stimulated glucose utilisation and this finding is consistent with the observation that resumption of full feeding enhanced insulin-induced glucose A-V concentration difference across the hind limb in sheep (Table 2). An interesting point is the increase in the concentration of blood glucose in animals in Groups I and II in Period II. Such an increase could be reflected in an increase in SSGIR as previously discussed.

Arterial Glucose, Glucose Arterio Venous (A-V) Concentration Difference and Glucose Extraction

Mean values of arterial glucose concentrations, glucose A-V concentration difference and glucose extraction by hind-limb muscles at the end of Period I and Week 2 of Period II are presented in Tables 2. Means of glucose A-V concentration differences infused for Groups I and II tended to be higher than

Table 2. Mean ($\pm$ sem) steady state values of glucose infusion rate (SSGIR), arterial plasma glucose concentration, glucose arterio-venous (A-V) concentration difference, glucose extraction, blood flow and glucose uptake by hind-limb muscles of animals in Groups I (underfed and exercised), II (underfed and not exercised) and III (fully fed and not exercised) during Periods I and II

	Period I				Period II			
	I	II	III	P	I	II	III	P
SSGIR (mg/mU insulin infused/kg LW)	0.03 $\pm$ 0.004 ^{ab}	0.02 $\pm$ 0.002 ^b	0.03 $\pm$ 0.003 ^{al}	0.030	0.03 $\pm$ 0.003 ^a	0.04 $\pm$ 0.004 ^a	0.025 $\pm$ 0.002 ^b	0.025
Arterial (mM)								
Pre infusion	3.50 $\pm$ 0.18 ^a	3.54 $\pm$ 0.24 ^a	4.47 $\pm$ 0.15 ^b	0.040	4.27 $\pm$ 0.258	4.00 $\pm$ 0.192	4.29 $\pm$ 0.174	0.594
During infusion	3.94 $\pm$ 0.26	3.64 $\pm$ 0.41	4.84 $\pm$ 0.30	0.054	4.39 $\pm$ 0.234	4.21 $\pm$ 0.233	4.26 $\pm$ 0.167	0.830
A-V (μ mol/mU insulin infused/min/kg muscle)								
During infusion	1.28 $\pm$ 0.23	0.87 $\pm$ 0.16	0.79 $\pm$ 0.13	0.147	0.94 $\pm$ 0.06	1.08 $\pm$ 0.11	0.70 $\pm$ 0.202	0.186
Extraction (%/kg)								
Pre infusion	0.84 $\pm$ 0.16	0.83 $\pm$ 0.15	0.69 $\pm$ 0.07	0.538	0.78 $\pm$ 0.10	0.92 $\pm$ 0.16	0.63 $\pm$ 0.13	0.181
During infusion	4.71 $\pm$ 0.90	3.70 $\pm$ 0.72	3.49 $\pm$ 0.54	0.478	3.79 $\pm$ 0.26	3.88 $\pm$ 0.39	2.9 $\pm$ 0.41	0.064
Blood flow during infusion (mL/min/100 g muscle)	9.79 $\pm$ 1.09	9.13 $\pm$ 0.84	11.97 $\pm$ 1.13	0.161	11.59 $\pm$ 0.67	12.35 $\pm$ 0.96	11.43 $\pm$ 0.99	0.742
Glucose uptake during infusion (μ mol/min/100 g muscle)	2.78 $\pm$ 0.44 ^a	2.06 $\pm$ 0.38 ^a	3.97 $\pm$ 0.34 ^b	0.018	3.26 $\pm$ 0.25	3.36 $\pm$ 0.43	3.04 $\pm$ 0.54	0.911

^{ab} Means with the different superscript in the same row are different at $P < 0.05$ significantly

those for Group III when they are expressed per kg hind-limb muscle per mU insulin.

Due to significant differences in live weight (LW) between Group III and Groups I and II, discussion on glucose A-V concentration difference across the hind-limb muscle is made on the basis per mU insulin infused per kg. The trends in glucose A-V concentration difference and glucose extraction were similar.

At the end of Period I, mean values of glucose A-V concentration difference, during insulin infusion, for Groups I and II were 1.6 and 1.1 times that for Group III. Animals in Group I tended to have higher mean values of glucose A-V concentration difference than animals in Group III ($P=0.07$). It might be concluded from this finding that exercise had an additive effect on insulin-increased glucose A-V concentration difference across hind-limb muscles of underfed animals. Exercise is likely to increase distribution of fast twitch oxidative muscle fibres which are known to enhance insulin sensitivity (Kriketos *et al.*, 1996).

Resumption of full feeding resulted in increased mean values of glucose A-V concentration difference for Groups I and II which were 1.4 and 1.5 times higher ($P=0.186$) respectively, than those for the control animals in Group III. Such increases are consistent with observations of Yuwono (2000) that there was increased efficiency in N utilisation during resumption of full feeding by animals in Groups I and II. An increased glucose A-V concentration

difference by muscle of these animals probably was to support the increased utilisation of glucose in protein synthesis.

Blood Flow and Glucose Uptake

Mean values of blood flow across the hind limb and glucose uptake by the hind limb at the end of Period I and Week 2 of Period II are presented in Table 2. At the end of Period I and week 2 of Period II, mean values of blood flow across the hind-limb muscle were not statistically different amongst Groups. At the end of Period I, mean values of glucose uptake by the hind limb during infusion of insulin and glucose were significantly lower in animals in Groups I and II than those for animals in Group III. At week 2 of Period II, mean values of glucose uptake by the hind limb were not different amongst Groups.

It was found in Period I that glucose uptake was lower in animals in Groups I and II than for animals in Group III and this was due to low absolute values of glucose A-V concentration difference and blood flow. Blood flow values across hind-limb muscles of animals in Groups I and II were 19% and 24% lower respectively than those across the hind-limb muscle of animals in Group III. Low blood flow values probably reflect a reduction in metabolic rates in Groups I and II. Mahyuddin (1990) has reported a reduction in CO₂ entry rate (reflecting a reduction in metabolic rate) in underfed sheep.

When full feeding resumed for 14 days, mean values of glucose uptake by hind-limb muscle were not different amongst groups because absolute values of glucose A-V concentration difference and blood flow were similar across Groups. Bigdelli (1996) has reported similar results from his study.

Conclusions

When sheep were underfed, insulin sensitivity of hind-limb muscle was not different from that of fully fed animals. However, inclusion of exercise during underfeeding improved insulin sensitivity in hind-limb muscles. Resumption of full feeding improved insulin sensitivity of hind-limb muscles in previously underfed animals. The hind-limb muscles of previously underfed and exercised animals were still sensitive to insulin when exercise was terminated and after full feeding had resumed for 14 days.

Although hind-limb muscles of underfed and exercised animals and underfed but not exercised animals are more sensitive to insulin than those of fully fed sheep at the end of the underfeeding period, total glucose uptake by the hind-limb muscle was much lower in the former groups. Resumption of full feeding resulted in no apparent difference in total glucose uptake by the hind-limb muscle between sheep experiencing nutritional stress and being exercised compared with control sheep

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