Physiological glucocorticoid levels regulate glutamine and insulin-mediated glucose metabolism in skeletal muscle of the rat

Studies with RU 486 (mifepristone)

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This study examined the effects of antagonism of the peak level of glucocorticoids *in vivo*, which occurs as rats enter the feeding/activity (dark) period on glucose and glutamine metabolism in incubated isolated rat soleus muscle preparations. Thus the rats were treated with the potent glucocorticoid antagonist RU 486 2 h before and 1 and 2 h into the dark period. Both the content of glutamine in skeletal muscle *in vivo* and plasma glucose and glutamine concentrations were elevated midway through the dark period, compared with the beginning of the period. RU 486 prevented the increases in plasma glucose and glutamine and caused a significant decrease in both the rate of release of glutamine in soleus muscle *in vitro* and the content of glutamine in gastrocnemius muscle. The sensitivity of soleus muscle to insulin *in vitro* is markedly decreased when isolated midway through the dark period (i.e. at 03:00 h) [Leighton, Kowalchuk, Challiss & Newsholme (1988) *Am. J. Physiol.* **255**, E41–E45]. We now show that the concentrations of insulin required to stimulate lactate formation and glycogen synthesis half-maximally were 95 and 250 μ units/ml respectively, and treatment of rats with RU 486 decreased these values to 55 and 90 μ units of insulin/ml respectively. Thus antagonism of the action of the normal circadian rise in the level of glucocorticoids in rats reverses insulin insensitivity in soleus muscles *in vitro*.

INTRODUCTION

The sensitivities of the rates of lactate formation and glycogen synthesis to insulin are greatest in the incubated soleus muscle isolated from rats at either 09:00 h or 21:00 h [1]. Soleus muscles removed from rats at 16:00 and 03:00 h (approximately halfway through the light and dark periods respectively) exhibit marked insulin resistance [1]. Rats fed *ad libitum* consume most of their daily diet (> 80%) (also they are more active) during the dark period (20:00–08:00 h in the present study) (see Fig. 1) [2].

The altered response of glucose utilization to insulin in incubated soleus muscle isolated at 03:00 h is similar to that observed with muscles from glucocorticoid-treated rats [1]. Corticosterone is secreted from the adrenal cortex in a circadian fashion [3]: the highest plasma level of corticosterone occurs as rats enter the dark period [4], i.e. about 20:00 h. However, it has been established that a delay of at least 4 h exists between an increase in plasma glucocorticoid levels and the effect of the hormone on skeletal muscle [5], so that a similar time delay between peak levels of corticosterone *in vivo* and the effect on muscle could be the cause of the decrease in the response to insulin by skeletal muscle midway through the dark period. Surprisingly, to our knowledge, the effects of physiological concentrations of glucocorticoids on glucose metabolism in skeletal muscle have not been established. To carry out such a study, we administered a specific glucocorticoid-receptor antagonist (RU 486) to rats to block corticosterone action, and the effects on insulin-stimulated rates of lactate formation and glycogen synthesis in incubated soleus muscle preparations were investigated (for protocol see Fig. 1 and the Materials and methods section).

Treatment of rats with pharmacological doses of glucocorticoids causes an increase both in muscle glutamine synthetase activity [6,7] and in the rate of glutamine efflux from the hindlimb [8,9].

Consequently, the effects of RU 486 on glutamine content of muscle and on the rate of release of glutamine from incubated soleus muscle isolated at 03:00 h were also investigated.

MATERIALS AND METHODS

Animals and materials

Male Wistar rats (50–60 g) were purchased from Harlan-Olac (Bicester, Oxon, U.K.) and kept in the Department's animal house for at least 18 days before experimentation. The rats were housed under a controlled 12 h light/dark cycle and had access to food and water *ad libitum* until 1 h before being killed. Soleus muscles were isolated from animals (final body wts. 150–170 g) at times corresponding to 09:00 and 03:00 h (for this group dissection was carried out, at 15:00 h, on rats kept under reversed light/dark cycle conditions). The glucocorticoid antagonist RU 486 inhibits steroid-mediated effects by specifically binding to the glucocorticoid receptor, thus markedly decreasing the rate of transport of the steroid-receptor complex into the nucleus [10]. The effects of glucocorticoids in man *in vivo* are inhibited by administration of RU 486 at a time prior to the peak plasma levels of this hormone [11]. Animals were randomly divided into control and RU 486-treated groups [treated at 18:00, 21:00 or 22:00 h with a single intraperitoneal injection of RU 486 (50 mg/kg) dissolved in ethanol]. This dose of RU 486 gives full antagonist, but no agonist, activity [12]. Control rats received an injection of ethanol (0.2 ml). In the present study, rats were given RU 486 2 h before the beginning of the dark period (i.e. at 18:00 h), and some 9–10 or 15–16 h later the sensitivity of glycolysis and glycogen synthesis to insulin was determined (see Fig. 1). Administration of RU 486 to rats had no effect on food intake (values for control and treated rats were respectively 3.1 ± 0.4 and 3.0 ± 0.9 g of food/h per kg for three separate

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Fig. 1. Protocol for times when RU 486 was administered to rats and when rat soleus muscles were isolated

The time period when the highest plasma levels of corticosteroids are observed is denoted by the rectangular box. In the present study the dark period was between 20:00 h and 08:00 h.

observations). All reagents and biochemicals were obtained from sources previously given [13,14], except RU 486 (also known as RU 38486 and mifepristone), which was kindly given by Dr. D. Philibert of Roussel Uclaf, Romainville, France.

Incubation procedures

Immediately after killing, blood samples were taken from the heart in heparinized syringes and the gastrocnemius muscles were freeze-clamped in liquid N_2 . Blood was immediately centrifuged and plasma was stored at $-20^\circ C$ with 1000 i.u. of aprotinin/ml until assayed. Stripped soleus muscles were prepared as previously described [15,16]. This involved dissection of the muscle longitudinally, ligation of the distal and proximal tendons and attachment of the strips to stainless-steel holders, such that the muscle was maintained at 'in situ' resting tension. Muscle strips were then placed in 35 ml silicone-coated Erlenmeyer flasks containing Krebs-Henseleit bicarbonate buffer of the following composition: 120 mM-NaCl, 7 mM-Hepes, 25 mM- $NaHCO_3$, 4 mM-KCl, 1.1 mM- $CaCl_2$, 1.2 mM- KH_2PO_4 , 1.2 mM- $MgSO_4$, 5.5 mM-glucose, 4 mM-pyruvate. Defatted BSA was

added to a final concentration of 1% (w/v) and the pH was adjusted to 7.31. After a 30 min preincubation period at $37^\circ C$ and gassing with O_2/CO_2 (19:1), muscles were incubated in the above incubation medium (minus pyruvate) plus $[U-^{14}C]$ glucose (0.25 $\mu Ci/ml$) and various concentrations of insulin (see the Results section). The muscles were incubated for 1 h, gassed (O_2/CO_2 , 19:1) for the first 15 min in a shaking water bath, after which the muscle strips were removed, rapidly blotted and frozen in liquid N_2 . Lactate concentration in the medium [17] and the amount of $[U-^{14}C]$ glucose incorporated into glycogen [18] (glycogen synthesis) were measured.

For measurement of muscle amino acid contents, the frozen gastrocnemius muscle was powdered under liquid N_2 with a mortar and pestle. A portion of the powder was homogenized in ice-cold $HClO_4$ (10%, w/v) with a Polytron homogenizer. The homogenate was centrifuged (1500 g) for 8 min at $4^\circ C$ and the supernatant was neutralized with 10 mM-KOH (plus 40 mM-triethanolamine). Plasma was extracted with ice-cold $HClO_4$ (5%) and centrifuged (13000 g) for 10 min at $4^\circ C$. The supernatant was neutralized with 5 M-KOH (plus 7.7 mM-triethanolamine). The concentrations of glucose [19], glutamine [20], alanine [21] and branched-chain amino acids [22] were measured in neutralized extracts of muscle or plasma; the glutamine concentration was measured in samples of incubation medium that contained basal levels of insulin (i.e. 1 or 10 $\mu units/ml$). The rate of glutamine release from the muscle was calculated from the concentration present in the medium.

RESULTS

The rates of lactate formation and glycogen synthesis in response to various concentrations of insulin are given in Fig. 2. The concentrations of insulin required to stimulate lactate formation and glycogen synthesis half-maximally (EC_{50} values) in soleus muscle from control animals are 95 and 250 $\mu units/ml$ respectively. There are differences in responses in soleus muscles isolated at 03:00 h measured in the present study and a previous

Fig. 2. Effects of administration of RU 486 to rats at 18:00 h on insulin-stimulated rates of lactate formation and glycogen synthesis in incubated soleus-muscle preparations isolated at 03:00 h

The results are presented as means $\pm$ S.E.M. for at least 11 separate experiments. Statistically significant differences (non-paired Student's t test) between control ($\circ$) and RU 486-treated ($\bullet$) groups are indicated as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 1. Effects of administration of RU 486 to rats at 18:00 h, 21:00 h and 22:00 h on insulin-stimulated rates of lactate formation and glycogen synthesis in incubated soleus-muscle preparations isolated at 09:00 h

RU 486 was administered at the times indicated in the Table, and all soleus muscles were removed at 09:00 h. The results are presented as means $\pm$ S.E.M. for the numbers of observations given in parentheses. Statistically significant increases (non-paired Student's *t* test) between control and RU 486-treated groups are indicated as follows: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Insulin concn. (μ units/ml)	Rates of glycogen synthesis (μ mol of glucosyl units/h per g)			
	Control	RU 486		
		18:00 h	21:00 h	22:00 h
1	1.19 $\pm$ 0.12 (15)	3.27 $\pm$ 0.29 (7) ***	2.63 $\pm$ 0.26 (4) ***	2.32 $\pm$ 0.40 (4) *
10	1.70 $\pm$ 0.16 (14)	2.85 $\pm$ 0.25 (7) **	3.46 $\pm$ 0.25 (4) ***	2.83 $\pm$ 0.72 (4)
100	3.14 $\pm$ 0.29 (16)	4.28 $\pm$ 0.36 (8) *	5.15 $\pm$ 0.35 (4) *	4.42 $\pm$ 0.72 (4)
1000	5.22 $\pm$ 0.45 (7)	6.00 $\pm$ 0.38 (7)	—	—
10000	6.00 $\pm$ 0.42 (14)	7.49 $\pm$ 0.50 (7)	7.03 $\pm$ 0.38 (4)	4.82 $\pm$ 0.74 (4)

	Rates of lactate formation (μ mol of glucosyl units/h per g)			
	Control	RU 486		
		18:00 h	21:00 h	22:00 h
1	4.16 $\pm$ 0.26 (15)	5.87 $\pm$ 0.90 (7)	5.54 $\pm$ 1.00 (4)	2.99 $\pm$ 0.33 (4)
10	4.70 $\pm$ 0.27 (14)	6.01 $\pm$ 0.66 (7)	6.70 $\pm$ 0.66 (4) *	3.94 $\pm$ 0.73 (4)
100	6.27 $\pm$ 0.43 (15)	7.03 $\pm$ 0.50 (8)	7.76 $\pm$ 0.43 (4)	4.11 $\pm$ 0.34 (4)
1000	8.45 $\pm$ 0.63 (7)	8.39 $\pm$ 0.58 (6)	—	—
10000	8.73 $\pm$ 0.42 (15)	7.84 $\pm$ 0.53 (7)	8.58 $\pm$ 0.58 (4)	5.59 $\pm$ 0.79 (3)

Table 2. Effect of administration of RU 486 to rats at 18:00 h, 21:00 h and 22:00 h on glycogen content in incubated soleus muscle isolated at 03:00 h and 09:00 h

Results are presented for the numbers of observations given in parentheses.

Time (h)		Glycogen content (μ mol of glucosyl units/g wet wt.)	
Administration	Isolation	Control	RU 486
18:00	03:00	44.6 $\pm$ 4.2 (11)	34.1 $\pm$ 3.9 (10)
18:00	09:00	24.2 $\pm$ 0.9 (7)	24.2 $\pm$ 1.3 (7)
21:00	09:00	25.4 $\pm$ 1.0 (7)	21.0 $\pm$ 1.5 (8)
22:00	09:00	28.1 $\pm$ 1.6 (8)	26.0 $\pm$ 1.8 (8)

study reported from our laboratories [1]. There was a greater responsiveness of the rates of glycogen synthesis to sub-maximal and maximal concentrations of insulin (i.e. 100, 1000 and 10000 μ units/ml). Consequently, the calculated concentration of insulin required to stimulate glycogen synthesis half-maximally (i.e. EC_{50} value) is lower (i.e. 250 in the present study versus 493 [2] μ units/ml). This is a significant improvement in the sensitivity of glycogen synthesis to insulin. Nevertheless, since the response (i.e. 2.2 μ mol/h per g) of glycogen synthesis to a physiological concentration of insulin (i.e. 100 μ units/ml) in muscles isolated at 03:00 h in the present study is significantly lower than the response in muscles isolated at 09:00 h, this clearly demonstrates that the muscles are insulin-resistant. It was not possible, in the present study, to determine whether the sensitivity of glycolysis

to insulin is decreased in incubated muscles isolated at 03:00 h as compared with 09:00 h [2]. This is due to lower rates of glycolysis and a smaller response of this process to insulin in soleus muscles isolated from fed rats as compared with muscles isolated from fasted rats [1,13,15].

The administration of RU 486 to rats at 18:00 h increased the rates of glycogen synthesis in soleus muscle isolated at 03:00 h, but not those of lactate formation, at a basal level of insulin (i.e. 1 μ unit/ml). However, an increase in the rates of both lactate formation and glycogen synthesis was observed at a sub-maximal concentration of insulin (i.e. 100 μ units/ml; see Fig. 2). The insulin EC_{50} values were decreased markedly for both lactate formation (55 μ units/ml) and glycogen synthesis (90 μ units/ml) after administration of RU 486. Hence it is concluded that administration of the glucocorticoid antagonist RU 486 results in an increase in the sensitivity of glycogen synthesis to insulin.

We investigated whether administration of RU 486 after the highest plasma level of corticosterone (i.e. at 21:00 and 22:00 h) had any effect on basal and insulin-mediated glucose metabolism in soleus muscle *in vitro*. The administration of RU 486 at 18:00 h significantly increased the basal (i.e. at 1 and 10 μ units of insulin/ml) and insulin (100 μ units/ml)-stimulated rates of glycogen synthesis in soleus muscle isolated at the time normally used for most incubation studies (i.e. 09:00 h; see Table 1). Similar results were obtained when rats were treated with RU 486 at 21:00 h and the soleus muscle was isolated at 09:00 h. However, with this treatment a significant increase in the rate of glycolysis was observed with 10 and 100 μ units of insulin/ml. Administration of RU 486 at 22:00 h significantly increased the rate of glycogen synthesis at only one insulin concentration (i.e. 1 μ unit/ml). However, the rates of glycogen synthesis tended to be higher at both 10 and 100 μ units of insulin/ml. Treatment of rats with RU 486 did not alter the insulin EC_{50} value for glycogen

Table 3. Effects of administration of RU 486 to rats at 18:00 h on plasma levels of glucose, glutamine, alanine and branched-chain amino acids

Values are means $\pm$ S.E.M. for at least 5 separate observations. Statistically significant differences (non-paired Student's *t* test) are indicated by : ^a*P* < 0.05 for comparison of 09:00 h group with 03:00 h group; ^b*P* < 0.05 for comparison of 03:00 h control group with RU 486-treated group.

	Plasma concentrations (mM)		
	Control (09:00 h)	Control (03:00 h)	RU 486 (03:00 h)
Glucose	8.61 $\pm$ 0.30	9.97 $\pm$ 0.58 ^a	8.26 $\pm$ 0.47 ^b
Glutamine	0.88 $\pm$ 0.07	1.06 $\pm$ 0.04 ^a	0.90 $\pm$ 0.04 ^b
Alanine	0.73 $\pm$ 0.03	0.52 $\pm$ 0.03 ^a	0.43 $\pm$ 0.04
Branched-chain amino acids	0.40 $\pm$ 0.02	0.37 $\pm$ 0.01	0.26 $\pm$ 0.01 ^b

synthesis compared with controls (EC_{50} value was 130 μ units/ml). Administration of RU 486 did not significantly decrease the glycogen content in incubated soleus muscle (Table 2). Lower basal and insulin-stimulated rates of lactate formation were measured in muscles isolated at 22:00 h. Since the combination of rates of lactate formation and glycogen synthesis are a good index of glucose disposal in isolated soleus-muscle preparations [23], this suggests that RU 486 administered at 22:00 h did not improve glucose disposal in soleus muscles isolated at 09:00 h. It should be noted that the rate of lactate formation in soleus muscles isolated at 09:00 h from control rats at 1 μ unit (but not 10 μ units, both of which are basal concentrations) of insulin/ml was lower than a previously reported value [1].

In untreated rats the plasma levels of glucose (16%) and glutamine (20%) were elevated and the level of alanine (29%) was decreased at 03:00 h compared with 09:00 h, which is the time when most experiments of this kind are routinely performed (Table 3). Administration of RU 486 decreased the plasma levels of glucose (17%), glutamine (15%) and branched-chain amino acids (30%) at 03:00 h, but had no effect on plasma alanine levels (Table 3). Similarly, the level of glutamine in the gastrocnemius muscle was elevated at 03:00 h compared with 09:00 h in untreated animals (Table 4). Administration of RU 486 caused a decrease in the content of glutamine (15%), but had no effect on the contents of either alanine or branched-chain amino acids (Table 4). Interestingly, the rate of release of glutamine from

the soleus muscle was significantly decreased (12%) after administration of RU 486 (Table 4).

DISCUSSION

Recently it was reported that the sensitivity of glucose metabolism to insulin in rat skeletal muscle changes markedly during the 24 h period of a normal day [1]. In contrast, there were only small changes in the plasma levels of insulin [1]. This suggested that at certain periods of the day skeletal muscle can be either sensitive or resistant to the effects of insulin. Insulin-resistance is defined as a condition in which a normal amount of insulin produced a subnormal biological response. Perhaps the regular diurnal variation of insulin-sensitivity is produced by fluctuations in the blood levels of other counter-regulatory hormones.

There is evidence that glucocorticoids can bring about decreased sensitivity of skeletal muscle to insulin: resistance to insulin is observed in rats chronically treated with dexamethasone [23] and in obese animal models (such as obese (*ob/ob*) mice [24] and Zucker (*fa/fa*) [25] and ventromedial-hypothalamic-lesioned (VMH) rats [26]); adrenalectomy of obese animals improves the sensitivity of glucose metabolism to insulin in skeletal muscle [24,25,27]; treatment of the adrenalectomized obese rats with glucocorticoids induces an insulin-resistant state [27,28]; and there is no circadian rhythm of plasma levels of corticosterone in obese animals [29–31]. Consequently, it is possible that marked increases in the plasma level of glucocorticoids, which occur in normal rats at the onset of the dark period, could be responsible for the insensitivity of glucose metabolism to insulin previously observed in skeletal muscle [1].

It has been established that administration of RU 486 to rats, at least 1 h before treatment with glucocorticoids, abolishes the effects of these hormones normally observed some hours later [12]. In the present work, therefore, rats were treated 2 h before the dark period. The responses of the rates of lactate formation and glycogen synthesis to various concentrations of insulin were measured about 9 h after administration of RU 486. From these measurements it was clear that the decrease in sensitivity to insulin of the isolated soleus muscle observed at 03:00 h in rats was largely abolished by treatment of the experimental animal with RU 486 (see Fig. 2). In addition, the calculated rates of glucose utilization (sum of the rates of lactate formation plus glycogen synthesis) in soleus muscle from RU 486-treated animals were increased at physiological concentrations of insulin (i.e. 10–100 μ units/ml; results not shown). Thus blockade of corticosterone action at the onset of the feeding/activity cycle in the rat improved the sensitivity of glucose utilization to insulin in skeletal muscle midway through this period. Antagonism of

Table 4. Effects of administration of RU 486 on rates of glutamine release from isolated incubated soleus muscle and contents of glutamine, alanine and branched-chain amino acids in gastrocnemius muscle

Results are presented as means $\pm$ S.E.M. for the numbers of replicate experiments given in parentheses. Statistically significant differences (non-paired Student's *t* test) between control values at 09:00 h and other values are indicated by : ^a*P* < 0.05, and between control (03:00 h) and other values are indicated by ^b*P* < 0.05. Certain data in this Table appeared in Table 2 of ref. [51] and are included here for comparison.

Condition	Rate of glutamine release (nmol/min per g)	Content (nmol/g)		
		Glutamine	Alanine	Branched-chain amino acids
Control (09:00 h)	—	4553 $\pm$ 185 (10)	2094 $\pm$ 101 (10)	402 $\pm$ 24 (10) ^b
Control (03:00 h)	41.8 $\pm$ 1.6 (24)	5373 $\pm$ 274 (20) ^a	1989 $\pm$ 120 (18)	317 $\pm$ 25 (5)
RU 486 (03:00 h)	36.8 $\pm$ 1.7 (26) ^b	4381 $\pm$ 254 (24) ^b	1800 $\pm$ 25 (20)	245 $\pm$ 23 (5) ^a

corticosterone effects either 2 h before or 1 h into the dark cycle gave a greater response of glycogen synthesis to physiologically relevant concentrations of insulin (see Table 1). In essence, this is an amelioration of insulin-resistance. There may be a smaller effect of RU 486 on the basal and insulin-stimulated response of glucose metabolism in muscles isolated at 09:00 h from rats administered with RU 486 at 22:00 h, as the combination of the rates of glycolysis and glycogen synthesis was not different from control values (see Table 1). However, these animals treated with RU 486 exhibited a blunted response to insulin. It is possible that RU 486 administered at 22:00 h is still effective at modulating insulin responses and that these sets of results may be anomalous compared with the 18:00 and 21:00 h time points. These findings provide evidence for the suggestion that physiologically relevant levels of glucocorticoids *in vivo* modulate the sensitivity of glucose metabolism to insulin in normal rats, and this may have significance in control of the blood glucose in rats during the circadian cycle [1]. A similar mechanism may exist in man, since glucose tolerance is decreased in the afternoon (equivalent to the dark period in rats) compared with that in the morning [32,33].

The mechanism by which RU 486 improves insulin-sensitivity (chiefly on the process of glycogen synthesis) is not known. At this stage we can only speculate on this mechanism. Although unlikely, the improvement of insulin-sensitivity may be caused by antagonism of progesterone effects. Glucocorticoids increase the content of glycogen in skeletal muscle [23,34] and some believe that the level of glycogen in muscle regulates the rate of glycogen synthesis [35]. So, if RU 486 dramatically decreased glycogen content in isolated incubated soleus muscle, this may lead to improved insulin responses. We did not find any evidence in support of this suggestion in the present study. Prostaglandins of the E series are known to increase insulin-sensitivity in soleus-muscle preparations *in vitro* [14]. Administration of dexamethasone to rats decreases insulin-sensitivity in muscle *in vitro* and decreases the rate of prostaglandin release and the prostaglandin content in skeletal muscle [36]. However, prostaglandins of the E series modulate only the sensitivity of glucose transport and lactate formation (and not glycogen synthesis) to insulin [14,36]. It is tempting to speculate that modulation of the level of agents that decrease the rate of basal and insulin-mediated glycogen synthesis may cause the effects produced by administration of RU 486 to rats. These agents include adrenaline and its analogues [37,38], the neuropeptide calcitonin-gene-related peptide [39,40] and the novel pancreatic β -cell hormone amylin [40–44]. Recently, it has been shown that dexamethasone treatment of rats dramatically increases amylin mRNA levels in the pancreas [45]. If glucocorticoids do regulate, at least in part, amylin (or adrenaline or calcitonin-gene-related peptide) homeostasis, then administration of RU 486 may interfere with the effects exerted by these agents on skeletal-muscle glucose metabolism *in vivo*. The findings from the present study suggest that an investigation of the alterations in the plasma levels of insulin, amylin, calcitonin-gene-related peptide and glucocorticoids in control and RU 486-treated animals is warranted. Glucocorticoids may also alter the level of insulin in the blood. Therefore, changes in the level of insulin *in vivo* in response to RU 486 may also play a role in modulation of glucose metabolism in muscle.

Evidence in support of the hypothesis that physiologically relevant levels of corticosterone influence amino acid metabolism in skeletal muscle has been provided by our study of glutamine metabolism. It has been shown previously that the administration of dexamethasone to rats increases glutamine synthase activity in skeletal muscle *in vitro* and that this effect is prevented by RU 486 [46,47]. However, RU 486 alone had no effect on glutamine synthetase activity [8]. Furthermore, administration of high doses

of glucocorticoids increases the rate of glutamine efflux from the limbs of rats [10], dogs [11], sheep [48] and man [49,50]. The present work provides evidence, for the first time, that variations in physiological levels of glucocorticoids influence the rate of glutamine release from skeletal muscle *in vitro*. Also, both skeletal-muscle and plasma glutamine levels are elevated at 03:00 h compared with 09:00 h. However, administration of RU 486 decreased muscle and plasma glutamine concentrations at 03:00 h to levels similar to those observed at 09:00 h.

In conclusion, the results from the present study provides evidence that physiologically relevant levels of corticosteroids are important regulators of both glucose and glutamine metabolism in skeletal muscle *in vitro* and probably *in vivo*.

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