Effects of fasting on blood plasma levels, metabolism and metabolic effects of epinephrine and norepinephrine in steers

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Abstract. Experiments were designed to study effects of 3 days of fasting on blood plasma levels, metabolic clearance rates (MCR) and effects of norepinephrine (NE) and epinephrine (E) on levels of glucose, non-esterified fatty acids (NEFA) and immunoreactive insulin (IRI) in 12 steers. During fasting, levels of E, NE and protein did not change, whereas IRI, T₃ and glucose decreased and NEFA, acetocetate and β-hydroxybutyrate increased. Before and at the end of fasting, NE or E were iv infused for 120 min. NE and E were elevated after 15 min and to the end of the infusion. The increase in E, but not in NE, was significantly greater after 3 days of fasting than before fasting (P < 0.05). MCR for E was lower after fasting (299 ± 17 vs 204 ± 10 ml·kg⁻⁰.⁷⁵·min⁻¹; P < 0.001), whereas MCR for NE was not significantly different (455 ± 37 vs 400 ± 27 ml·kg⁻⁰.⁷⁵·min⁻¹). MCR was higher for NE than for E, both before and after fasting (P < 0.05). After the infusions, E and NE decreased within minutes to pre-infusion concentrations. During E infusions, NEFA increased significantly more, whereas glucose increased less in fasted than in fed animals. During NE infusions, NEFA increased in fasted, but not in fed animals, and glucose increased less at the end than before fasting. IRI decreased during E infusions only in fed animals, and transiently increased after the infusion, except after NE infusion in fasted steers. Changes in plasma levels, clearance rates and sensitivity to effects of NE and E, together with alterations of insulin and T₃ concentrations, may contribute to shifts in energy metabolism during food restriction.

Blood levels of non-esterified fatty acids (NEFA) and ketone bodies are increased, whereas those of glucose are decreased during energy deficiency in cattle, as in other species. Epinephrine (E) and norepinephrine (NE) enhance lipolysis and glycogenolysis in steers, thereby raising circulating levels of NEFA, glycerol, glucose and lactic acid (Blum et al. 1982). The effect of exogenous E on NEFA and glycerol was enhanced, whereas it was reduced on glucose and lactic acid after 5 days of fasting (Blum et al. 1982). During E infusions, levels of E increased more markedly during than before fasting, suggesting reduced clearance of the hormone (Blum et al. 1982). On this basis we have studied more closely the behaviour of basal E and NE concentrations, kinetics and metabolic effects not only of E but also of NE, together with changes of immunoreactive insulin (IRI), before and during food restriction in steers.

Materials and Methods

Twelve steers (Schweizer Braunvieh, Simmental and Holstein), weighing 398 to 478 kg, were used. Experiments were always performed with 2 animals at the same time. Catheters were implanted into the jugular veins on the evening before the start of the 4-day experiments. On the first day, five blood samples were obtained from 08.00 to 09.00 h whereafter either NE or E (0.38 or 0.33 nmol·kg⁻¹·min⁻¹) were iv infused for 120 min, each in 6 of the 12 animals. Up to 13.00 h of the first day the animals received corn-silage, hay and concentrates according to requirements. Then, food
was completely removed for 3 days, whereas water was provided ad libitum. During this period body weight was reduced by 8.8%. Five blood samples were again obtained from 08.00 to 09.00 h on the second, third and fourth day of the experiments, followed on the fourth day by infusions of the same amounts of NE and E for 120 min, as before fasting. NE- or E-bitartrate was dissolved immediately before the infusion and kept in light-protected bottles in an ice-bath.

Blood samples (five samples obtained from 08.00 to 09.00 h and samples obtained before, during and after the infusions) were immediately transferred to tubes containing heparin (50 U-USP/ml blood) or ice-cold 0.7 mol/l perichol acid (1 ml per 1 ml blood) and centrifuged at 4°C within 2 h after collection. Plasma aliquots of heparinized blood samples were then transferred to polystyrol cups and frozen at −20°C until analysed for E, NE IRI, T3, NEFA, glucose and protein. Deproteinized supernatants were filtered and then frozen at −20°C until analysed for the determination of acetooacetate (AAC) and β-hydroxybutyrate (BHB). Catecholamines were determined radioenzymatically (Da Prada & Zürcher 1979). This assay was used previously for catecholamine determinations in plasma of cattle (Blum et al. 1982, 1985). Concentrations of IRI, T3, glucose, NEFA, BHB, AAC and protein were determined as previously described (Blum et al. 1985). All samples from one experiment were determined within the same assay. Control sera were measured to evaluate inter-assay variations for all parameters.

Data are expressed as means ± SEM. Calculations of changes during the 3 days of fasting, related to prefasting levels, were based on the mean of the 5 samples taken each day from 08.00 to 09.00 h. Basal levels before the infusions were calculated from the mean of 3 samples. Values of NE, E, IRI, NEFA, glucose and protein of each blood sample, obtained after the start of the infusions before or after fasting, were compared with mean basal levels. Comparisons of changes of the various parameters during the 120 min infusions were based on calculations of the area under the concentration curves (concentration·volume−1·120 min) for each individual. The metabolic clearance rate (MCR) for NE and E was calculated for the last 90 min of the infusions (MCR = NE or E infused per min over plasma concentration of NE or E). Statistical analysis was done by use of variance analysis and paired t-test.

**Results**

**Blood levels of NE, E, IRI, T3, glucose, NEFA, AAC, BHB and protein before and during fasting for 3 days (Table 1)**

Levels of E during fasting were not different from prefasting concentrations, whereas NE decreased during fasting, but not significantly. E and NE during the 1-h blood sampling period prior to the 4-day experiment did not change significantly (not shown). IRI, T3 and glucose were markedly

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<th>Table 1. Changes of E, NE, IRI, T3, glucose, NEFA, BHB, AAC and protein during fasting.</th>
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<td>AAC (µmol/l)</td>
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<td>Protein (g/l)</td>
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¹ Means (± SEM) before and during fasting represent the average of values measured from each of the 12 animals at 08.00, 08.15, 08.30, 08.45 and 09.00 h.

² Variance analysis (effect of fasting and significance of difference from levels before fasting).

* *P* < 0.05; NS or no symbol, *P* > 0.05.
Changes in the levels of epinephrine, norepinephrine, immunoreactive insulin, glucose, non-esterified fatty acids and protein before, during and after infusions of epinephrine (0.33 nmol·kg⁻¹·min⁻¹ for 120 min), before (●●●) and after (○○○) 3 days of fasting of steers (N = 6).

Blood levels of NE, E, IRI, glucose, NEFA, and protein before, during and after E infusions before and after 3 days of fasting (Fig. 1)

Concentrations of E before the infusions in fed and fasted animals were practically identical. During the infusions, E markedly increased within 15 min, then remained elevated until the end of the infusion (P < 0.001). The increase in E during fasting was greater than before fasting (P < 0.05). Correspondingly, MCR was significantly higher before than after fasting (299 ± 17 and 204 ± 10 ml·kg⁻⁰·⁷⁵·min⁻¹; P < 0.001). E decreased towards basal levels within minutes after the infusions. NE slightly increased 60 min after the start of the E infusions in fasted (P < 0.05), but not in fed animals.

Basal IRI was lower after than before fasting (P < 0.05). During E infusions, IRI decreased in fed (P < 0.05), but not in fasted animals. After the
Changes in the levels of norepinephrine, epinephrine, immunoreactive insulin, glucose, non-esterified fatty acids and protein before, during and after infusions of norepinephrine (0.38 nmol·kg\(^{-1}\)·min\(^{-1}\) for 120 min), before (––––) and after (○–○) 3 days of fasting of steers (N = 6).

End of the infusions, IRI increased more markedly in fed than in fasted animals (P < 0.05), above pre-infusion levels. NEFA increased more markedly during E infusions in fasted than in fed animals (P < 0.01). During E infusions, glucose increased in a similar manner in fed and fasted animals, but the absolute concentrations reached were lower during than before fasting (P < 0.05). Basal protein levels were higher in fasted than in fed animals (P < 0.05), but protein did not change during E infusions.

Changes of NE, E, IRI, glucose, NEFA and protein before, during and after NE infusions before and after 3 days of fasting (Fig. 2).

Basal NE was lower after than before fasting (P < 0.05). During NE infusions, NE first rapidly increased, then remained elevated up to 120 min (P < 0.05) and decreased towards basal levels within minutes after the infusions (P < 0.001). Concentrations of NE during the infusions were lower than those of E during E infusions (P < 0.05, see Fig. 1), even though the amounts of NE infused were slightly higher. Levels tended to be higher in fasted than in fed animals during the
infusions, but the differences were not significant. As a consequence, MCR (455 ± 37 and 400 ± 27 ml·kg⁻⁰.⁷₅·min⁻¹, respectively) was not significantly different in fed and fasted animals. Concentrations of E before and during NE infusions were very similar in fed and fasted animals, but increased after the infusions in fed, but not in fasted animals (P < 0.05).

Concentrations of IRI before, during and after the NE infusions were lower in fasted animals (P < 0.05). IRI did not change significantly during, but transiently increased after the NE infusions (P < 0.01) in fed, albeit not in fasted animals. NEFA were higher before, during and after NE infusions in fasted than in fed animals (P < 0.01). NEFA increased during the NE infusions in fasted, but not in fed animals (P < 0.01) and then decreased. On the other hand, glucose was lower before, during and after the infusions, and during the infusion increased less, in fasted than in fed animals (P < 0.05). Protein was significantly higher after than before fasting (P < 0.05), but did not change in response to NE infusions.

**Discussion**

During fasting IRI, T₃, and glucose decreased, whereas NEFA, ketone bodies and protein increased, as shown previously (Blum et al. 1982). The increase in protein suggests a slight hemoconcentration during fasting. Under these experimental conditions, demonstrating typical and marked metabolic and endocrine adaptations to energy deficiency, E did not change and NE decreased only slightly, although not significantly. In long-term energy-deficient lactating dairy cows we have found decreased NE levels over a 24-h period (Blum et al. 1985), presumably as a consequence of reduced sympathetic activity (Jung et al. 1978; Landsberg & Young 1978; Becker 1983; Knehaus & Romsos 1983). On the other hand, there is evidence for enhanced release of E during fasting in rats (Young & Landsberg 1979). It may be that the decrease of glucose during fasting in our study was too small or occurred too slowly to elicit an enhanced release of E.

During infusions of E and NE, levels of E or NE first rapidly increased and then reached a plateau. Concentrations of E before the infusions were lower, but during infusions higher than those of NE, indicating that NE had the greater clearance rate.

Levels of E during the infusions were significantly higher in fasted than in fed animals, whereas those of NE were not significantly affected by fasting. As a consequence, MCR for E, but not for NE, was lower in fasted than in fed animals. Thus, it appears that fasting differently affected the metabolism of E and NE in steers, in accordance with studies in mice (Young & Landsberg 1979). The mechanisms underlying the difference are not clear. After termination of the infusions, both E and NE levels decreased within minutes to pre-infusion concentrations, demonstrating that clearing mechanisms of E and NE were still fully operating. The very fast normalization of plasma NE and E levels can be interpreted as a mechanism that rapidly reduces the exposition of target organs to these highly active compounds.

During infusion of E and NE, levels of IRI decreased in fed but not in fasted animals, despite of hyperglycemia. Insulin secretion is suppressed by E and NE through interaction with α-adrenergic receptors on B-cells (Smith & Porte 1976). IRI increased immediately after the end of the E and NE administration, obviously as a consequence of the fall of E and NE towards basal levels and dependent on the degree of hyperglycemia. Thus, the rise of IRI was greatest after E infusions in fed animals and smallest after NE infusions in fasted animals. It is known that IRI responses to glucose infusions in steers are markedly reduced during energy deficiency (Brockman 1978; Bossart et al. 1985).

The more marked increase in glucose level during infusions of E, as compared with that during infusions of NE, was presumably in part the consequence of higher amounts of circulating E than NE. However, the incremental changes in glucose during E infusions were similar in fed and fasted animals, despite of markedly higher E levels during fasting. In addition, the rise of glucose levels was smaller in fasted than in fed animals during NE infusions, although NE levels were similar. A reduced increase in glucose and lactate levels during the administration of E during fasting has been described previously in cattle (Blum et al. 1982). It is presumably associated with a reduced amount of glycogen stored in the liver during energy deficiency and possibly a decreased sensitivity of target cells to E and NE.

During E infusions, NEFA increased much more markedly in fasted than in fed animals, as
shown previously in cattle (Sidhu & Emery 1973; Blum et al. 1982). It can be argued that the greater responsiveness of NEFA to E was either the consequence of enhanced fat cell sensitivity to the effects of E or due to exposition of fat cells to higher amounts of circulating E. However, NEFA increased during NE infusions only in fasted and not in fed animals. Because NE levels were similar in fed and fasted animals, the more marked rise of NEFA during energy deficiency must have been the consequence of enhanced sensitivity of fat cells to NE. Lower levels of insulin presumably added to the increased lipolytic effects of E and NE.

In conclusion, this study shows no change of basal E and NE levels, a reduced clearance rate of E but not of NE during infusions, enhanced responses of NEFA, but a reduced increase in glucose during the administration of both E and NE after 3 days of fasting. Together with reduced circulating insulin and T₃, changes in plasma levels, metabolism and metabolic effects of E and NE presumably contribute to enhanced utilization of fat and reduced utilization of glucose as energy-yielding substrates in energy deficiency.

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References
