Pre- and postprandial changes in plasma hormone and metabolite levels and hepatic deiodinase activities in meal-fed broiler chickens

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The present study aimed to study the effects of food deprivation and subsequent postprandial changes in plasma somatotrophic and thyrotrophic hormone levels and focused on the inter-relationships between these hormonal axes and representative metabolites of the intermediary metabolism of meal-fed broiler chickens. Male broiler chickens (2 weeks old) were fed a meal of 40–45 g/bird per d for two consecutive weeks (food-restricted (FR) treatment). The daily allowance was consumed in about 30 min. At 4 weeks of age, FR chickens were killed at several time intervals (ten per sampling time) in relation to the daily food allowance: before feeding (about 23·5 h of food deprivation), and at 10, 20, 30 (end of feeding), 40, 50, 60, 90, 120 and 200 min after initiation of feeding. Birds fed *ad libitum* served as controls (*ad-libitum* (AL) treatment). Liver tissue was collected for deiodinase type I and type III activity measurements and blood samples for analysis of growth hormone (GH), insulin-like growth factor (IGF)-I, thyroxine (T_4), 3,3′,5-triiodothyronine (T_3), glucose, non-esterified fatty acids (NEFA), uric acid, triacylglycerol (TG) and lactate levels. Food deprivation caused a shift from lipogenesis to lipolysis and increased fatty acid turnover, a reduction in protein anabolism and reduced metabolic rate. Food intake was followed immediately by a pronounced increase in metabolic rate, initially mainly based on anaerobic mechanisms. Refeeding gradually reversed the fasting-induced alterations in plasma hormone and metabolite levels, but the time course of changes differed between metabolites, which clearly preceded those of the hormones investigated. The order of responsiveness after food provision were glucose > uric acid $\geq$ NEFA > lactate > TG for the plasma metabolites and GH > T_3 > T_4 > IGF–I for hormones. Based on these different postprandial time courses, several functional relationships are proposed. Glucose is believed to be the primary trigger for the normalisation of the effects of fasting on these plasma variables by restoring hepatic GH receptor capacity, as well as decreasing deiodinase type III activity.

Broiler chickens: Meal feeding: Hormones: Metabolites: Deiodinases

In poultry, the effects of food deprivation or fasting on endocrine functioning and intermediary metabolism are well documented (for reviews, see Okumura & Kita, 1999; Buyse *et al.* 2001*a*). In brief, fasting is associated with increased plasma growth hormone (GH), thyroxine (T_4), corticosterone, glucagon and some insulin-like growth factor (IGF) binding proteins and non-esterified fatty acid (NEFA) concentrations, whereas plasma 3,3′,5-triiodothyronine (T_3), IGF-I, insulin, uric acid and

triacylglycerol (TG) levels are reduced compared with those of their fed counterparts. Furthermore, hepatic GH receptor capacity and IGF-I gene expression are impaired in fasting animals. On the other hand, plasma IGF-II levels seem to be much less responsive to food deprivation.

Refeeding reverses these processes after concomitant opposite changes in endocrine functioning (Buyse *et al.* 2000, 2001*a*). However, data on the short-term dynamics or the time course of these changes is scanty. Such

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information is warranted, as more insight into the pre- and postprandial alterations in endocrine and metabolic mechanisms is needed if the physiological effects of quantitative food manipulation on the bird's (re)productive performance and welfare are to be understood. Indeed, a severe food restriction needs to be applied in broiler breeder stock in order to prevent excessive adult body weight and fatness and hence poor reproductive performance (Costa, 1981) and this practice may compromise the bird's welfare (Hocking *et al.* 1996).

We therefore initiated a series of studies in order to establish the short-term (within hours) alterations in the somatotrophic and thyrotrophic axes in meal-fed broiler chickens (Buyse *et al.* 2000). The major findings of this first study are summarised on p. 645. The experiment presented here is not merely a repetition of this first study, but aimed to identify interactions between the somatotrophic and thyrothrophic axes and some key metabolites of the carbohydrate, fat and protein metabolism in meat-type chickens subjected to daily meal feeding schedules as applied commercially. Indeed, there are few results available on the interactions between the endocrine system and intermediary metabolism in avian species.

Materials and methods

Chickens, management and experimental design

Male broiler chicks (Hybro, 1 d old) were obtained from a local hatchery (Euribrid, Aarschot, Belgium) and placed in an environmentally controlled room. The lighting schedule provided 23 h light per d and wood shavings were used as litter. Temperature was set at 36°C and gradually reduced by 1°C/d or 1°C/2 d until 22°C was reached at 36 d of age. A commercial broiler starter diet (12·1 MJ metabolisable energy and 222 g crude protein (N × 6·25)/kg; Hendrix nv, B-2770 Merksem, Belgium) and water were provided *ad libitum*. At 14 d of age, chickens were randomly divided in equal numbers in two separate floor pens. Chickens in one pen were provided with a broiler grower diet (12·7 MJ metabolisable energy and 214 g crude protein (N × 6·25/kg; Hendrix nv.) on an *ad libitum* basis (*ad-libitum*-fed, AL), whereas in the other pen, the daily grower diet allowance was limited to 40 and 45 g/bird per d during week 3 and 4 respectively. The food-restricted (FR) chickens consumed their daily food allowance in about 30 min. The food was provided at a fixed time (09.00 hours) each day. Water was available at all times. The compositions and calculated analyses of the broiler starter and grower diets are presented elsewhere (Buyse *et al.* 2001*b*).

At the age of 28 d, fifty FR chickens were killed at several time intervals (five per sampling time) in relation to the daily food allowance: before feeding (about 23·5 h of food deprivation) and at 10, 20, 30 (end of feeding), 40, 50, 60, 90, 120 and 200 min after initiation of feeding. In addition, at each sampling time, five AL chickens were also killed. The same experimental protocol was repeated the next day, in total ten chickens per food treatment and sampling time. The chickens were stunned and then killed by exsanguination, and a blood sample collected in a heparinised tube that was kept on ice. The liver was quickly removed and a piece was immediately frozen in liquid N_2 and then stored at −70°C until analysed. Blood samples were centrifuged and plasma stored at −20°C until assayed for hormone and metabolite content.

Plasma analyses

Plasma T_3 and T_4 levels were measured by radioimmunoassay as described by Darras *et al.* (1992). Intra-assay CV were 4·5 and 5·4 % for T_3 and T_4 respectively. Antisera as well as T_3 and T_4 standards were purchased from Byk-Belga (B-1000 Brussels, Belgium). Chicken GH was measured with a homologous radioimmunoassay as developed and validated by Berghman *et al.* (1988). The intra-assay CV was 4·0 %. Plasma IGF-I concentrations were measured following acid–ethanol cryo-precipitation using a heterologous radioimmunoassay previously validated for chicken plasma (Huybrechts *et al.* 1985). The intra-assay CV was 6·9 %. Plasma metabolite concentrations were determined with an automated spectrophotometer (Monarch®; Instrumentation Laboratories, B-1930 Zoventem, Belgium) by using commercial kits: glucose, lactate, TG, uric acid (Instrumentation Laboratories) and NEFA (Wako, Neuss, Germany). All measurements for each variable were run in the same assay in order to avoid inter-assay variability.

Hepatic deiodinase activities

Outer-ring deiodinase (D) type I and inner-ring D type III activities were measured in hepatic microsomal fractions as described by Darras *et al.* (1992). The final incubation conditions were 200 μg microsomal protein/ml with 1 μM-reverse T_3 as substrate (in the presence of 5 mM-dithiothreitol and 2 mM-EDTA) for D-I activity, and 1 mg microsomal protein/ml with 1 nM-T_3 as substrate (in the presence of 1 μM-reverse T_3, 0·1 mM-6-propyl-2-thiouracil, 50 mM-dithiothreitol and 2 mM-EDTA) for D-III activity.

Statistical analyses

Plasma hormone and metabolite data and tissue D-I and III activities were analysed by two-way ANOVA using the General Linear Models procedure of the SAS® system (version 6, 1986; Statistical Analysis Systems Institute Inc., Cary, NC, USA) with food treatment (AL or FR) and time of sampling as classification variables. Hepatic D-I and -III activities were log-transformed before statistical analysis in order to alleviate non-normality and to reduce heterogeneity of variance. In addition, one-factor ANOVA was used for analysis of time-dependent changes for each variable for each food treatment separately, followed by a multiple comparison of means by using the Scheffe test, as well as for food treatment differences as each sampling point. The non-transformed results are presented as mean values with their standard errors.

Results

Plasma hormone levels

The results of the ANOVA for the plasma hormone, metabolite levels and hepatic D-I and -III activities are summarised in Table 1.

FR chickens were characterised by significantly ($P<0.0001$, Table 1) higher plasma GH levels during the entire sampling session compared with the levels of AL chickens (Fig. 1). This was especially the case in 23.5 h FR chickens and up to 50 min after initiation of the meal. A significant ($P=0.0008$) effect of time was observed for the FR but not for AL chickens, resulting in a significant time × food treatment interaction ($P=0.0005$). Indeed, the pooled plasma GH levels for the first 50 min (81.6 (SE 5.4) ng/ml, n 60) were significantly higher ($P=0.007$) than the pooled GH levels (47.3 (SE 4.9) ng/ml, n 40) of the next 140 min, indicative of a postprandial decrease in plasma GH concentrations of FR chickens.

Imposing meal feeding caused an overall reduction in circulating IGF-I levels ($P<0.0001$). Food deprivation for 23.5 h caused a significant ($P<0.05$) decrease in plasma IGF-I levels and this remained so during the 200 min sampling period (Fig. 2). Plasma IGF-I levels showed significant variations with time ($P=0.0002$) for both treatments, although more pronounced for FR (time effect $P<0.0001$) than for AL chickens (time effect $P=0.017$), resulting in a trend towards a food treatment × time interaction ($P=0.085$).

FR resulted in a marked increase ($P<0.0001$) in plasma T_4 levels (Fig. 3). Plasma T_4 levels of FR chickens remained significantly higher ($P<0.001$) than those of AL chickens during the entire sampling period. Plasma T_4 levels of FR chickens changed very slowly, as only after 200 min a significant ($P<0.05$) decrease was observed compared with prefeeding level. As a consequence, a significant ($P<0.0001$) time effect was observed for FR chickens, whereas this was not the case for AL chickens, resulting in a significant ($P=0.0125$) food treatment × time interaction.

In contrast, plasma T_3 levels of FR chickens were significantly lower ($P<0.0001$) compared with those of AL chickens (Fig. 4). When feeding was initiated, plasma T_3 levels rose gradually and were not different from AL values after 200 min. Plasma T_3 levels of AL chickens did not fluctuate during the sampling session, resulting in a significant ($P<0.0001$) food treatment × time interaction.

Plasma metabolites

Food deprivation induced a pronounced decrease in circulating glucose levels ($P<0.001$, Fig. 5). However, within 10 min of refeeding, plasma glucose concentrations rose to the level of AL chickens, followed by a significant overshoot for the next 30 min. After a temporary decrease, plasma glucose levels of FR chickens rose again so that they were greater than values of AL chickens at 120 and 200 min.

The absence of food for a prolonged time reduced circulating TG levels by half ($P<0.001$, Fig. 6). After meal termination, plasma TG levels started to increase gradually and were significantly ($P<0.05$) higher than prefeeding levels after 60 min. Similar levels as those of AL chickens were reached 120 min after meal initiation.

There were no differences in plasma NEFA levels between FR and AL chickens (Fig. 7). Plasma NEFA levels dropped sharply during the first 20 min after initiation of feeding, remained at this basal level for 20 min and then started to increase again during the next 10 min in order to reach AL levels at 50 min after food provision.

Plasma lactate levels were significantly ($P<0.001$) reduced by food deprivation (Fig. 8). After food was presented, plasma lactate levels of FR chickens gradually increased until AL levels were reached within 30 min.

Food deprivation significantly ($P<0.001$) reduced plasma uric acid levels (Fig. 9). After food was introduced, plasma uric acids doubled after 10 min and reached AL values by the termination of the meal. Between 60 and

Table 1. Probability values for feeding regimen (*ad-libitum* or food-restricted) and time of sampling and their interactions, and the effect of sampling time within each food programme*

	Statistical significance of effect (ANOVA): P				
Variable	Feeding regimen	Time	Feeding×time	Time × AL regimen	Time × FR regimen
Plasma					
Growth hormone	<0.0001	0.0017	0.0005	NS	0.0008
Insulin-like growth factor-I	<0.0001	0.0002	0.085†	0.0177	<0.0001
Thyroxine	<0.0001	0.0023	0.0125	NS	<0.0001
Triiodothyronine	<0.0001	<0.0001	<0.0001	NS	<0.0001
Glucose	0.0005	<0.0001	<0.0001	NS	<0.0001
Triacylglycerol	<0.0001	<0.0001	<0.0001	NS	<0.0001
NEFA	<0.0001	<0.0001	0.0089	NS	<0.0001
Lactate	<0.0001	0.0027	0.0162	NS	0.0002
Uric acid	NS	0.0008	<0.0001	0.0018	<0.0001
Liver					
D-I	NS	0.0234	<0.0001	NS	0.0002
D-III	<0.0001	<0.0001	<0.0001	NS	<0.0001

AL, *ad-libitum* fed; FR, food-restricted; NEFA, non-esterified fatty acid; D, deiodinase.
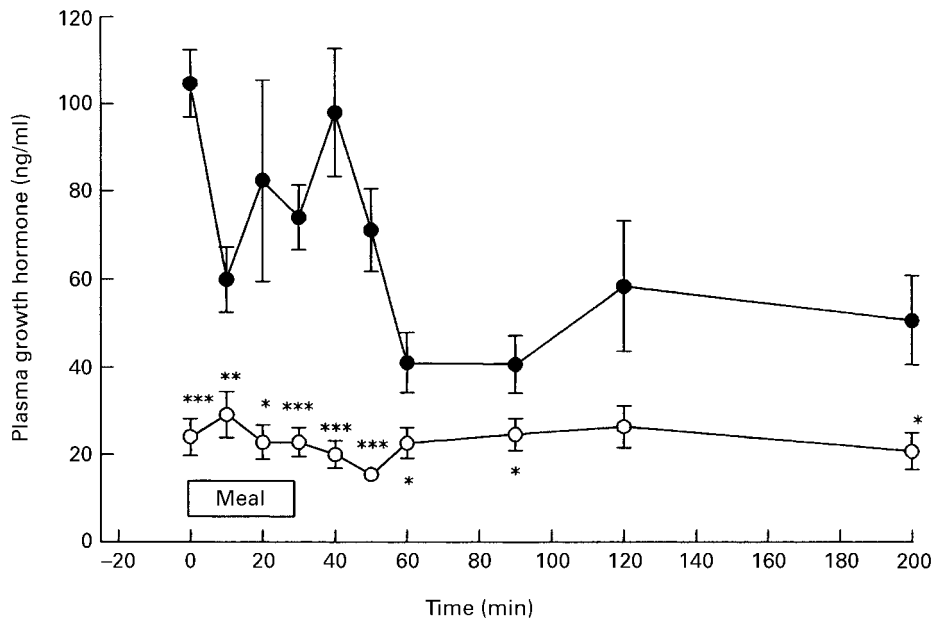* For details of diets and procedures, see p. 642.
† $P>0.05$.

Fig. 1. Plasma growth hormone concentrations immediately before, during and after a 30 min meal in male broiler chickens fed *ad libitum* (○) or food-restricted (●). Values are means with their standard errors (*n* 10). For details of diets and procedures, see p. 642. Mean values were significantly different from food-restricted male broiler chickens within each sampling time: *$P<0.05$, **$P<0.01$, ***$P<0.001$.

120 min after the meal was provided, plasma uric acid levels of FR chickens were significantly ($P<0.01$) higher than those of AL chickens, but this was due to a temporary drop in the uric acid levels of the latter chickens.

With the exception of uric acid, there were no significant time fluctuations in plasma metabolite levels of AL chickens, resulting in significant food treatment × time interactions for these plasma variables (Table 1).

Hepatic deiodinase activities

Food deprivation caused a significant ($P<0.001$, Fig. 10) decrease in *in vitro* hepatic D-I activity. When food was provided, hepatic D-I activities returned to values for AL chickens within 10 min. As a consequence of this quick normalisation, there was no effect of food treatment on D-I activity. A significant time effect was observed for
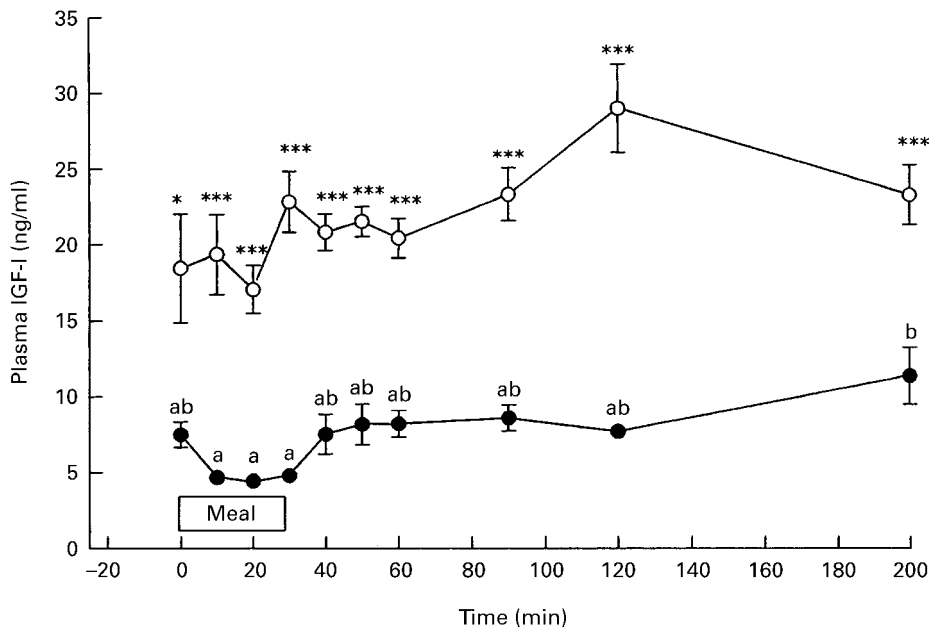

Fig. 2. Plasma insulin-like growth factor-I (IGF-I) concentrations immediately before, during and after a 30 min meal in male broiler chickens fed *ad libitum* (○) or food-restricted (●). Values are means with their standard errors (*n* 10). For details of diets and procedures, see p. 642. [a,b]Mean values with unlike superscript letters were significantly different between the sampling times of food-restricted chickens (Scheffe test, $P<0.05$). Mean values were significantly different from food-restricted male broiler chickens within each sampling time: *$P<0.05$, ***$P<0.001$.
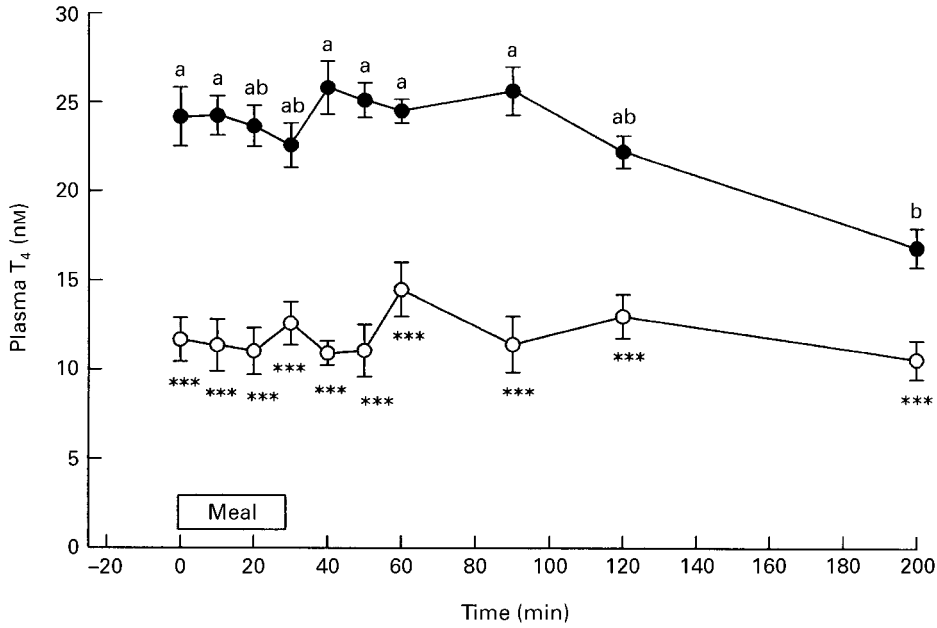
Fig. 3. Plasma thyroxine (T_4) concentrations immediately before, during and after a 30 min meal in male broiler chickens fed *ad libitum* (○) or food-restricted (●). Values are means with their standard errors (*n* 10). For details of diets and procedures, see p. 642. [a,b]Mean values with unlike superscript letters were significantly different between the sampling times of food-restricted chickens (Scheffe test, $P < 0.05$). Mean values were significantly different from food-restricted male broiler chickens within each sampling time: ***$P < 0.001$.

FR ($P = 0.0002$), but not for AL chickens, giving rise to a significant food treatment × time interaction ($P < 0.0001$).

In contrast, *in vitro* hepatic D-III activity was increased 5-fold by food deprivation, and remained higher than values for AL chickens during almost the entire sampling session ($P < 0.0001$; Fig. 11). A decrease in D-III activity in FR chickens was observed after about 60 min, but an entire normalisation to values for AL chickens was only completed after 200 min.

Discussion

Food deprivation

The present observed effects of food deprivation on plasma somatotrophic and thyrotrophic hormone levels are in accordance with those of our previous study (Buyse *et al.* 2000). In brief, we found that, compared with their AL counterparts, 4-week-old FR chickens (access to food for 30 min/d for 2 weeks) were characterised by higher mean
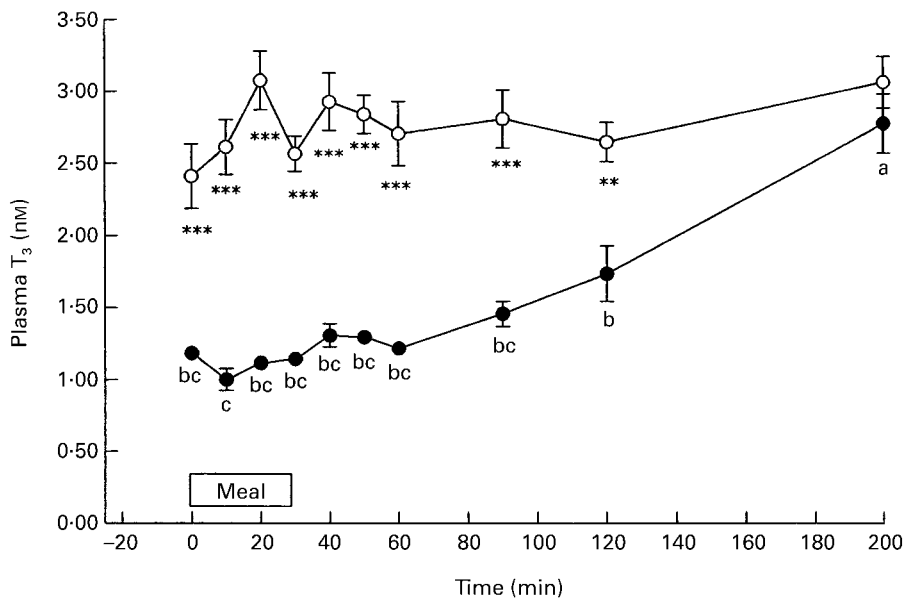

Fig. 4. Plasma 3,3′,5-triiodothyronine (T_3) concentrations immediately before, during and after a 30 min meal in male broiler chickens fed *ad libitum* (○) or food-restricted (●). Values are means with their standard errors (*n* 10). For details of diets and procedures, see p. 642. [a,b,c]Mean values with unlike superscript letters were significantly different between the sampling times of food-restricted chickens (Scheffe test, $P < 0.05$). Mean values were significantly different from food-restricted male broiler chickens within each sampling time: **$P < 0.01$, ***$P < 0.001$.
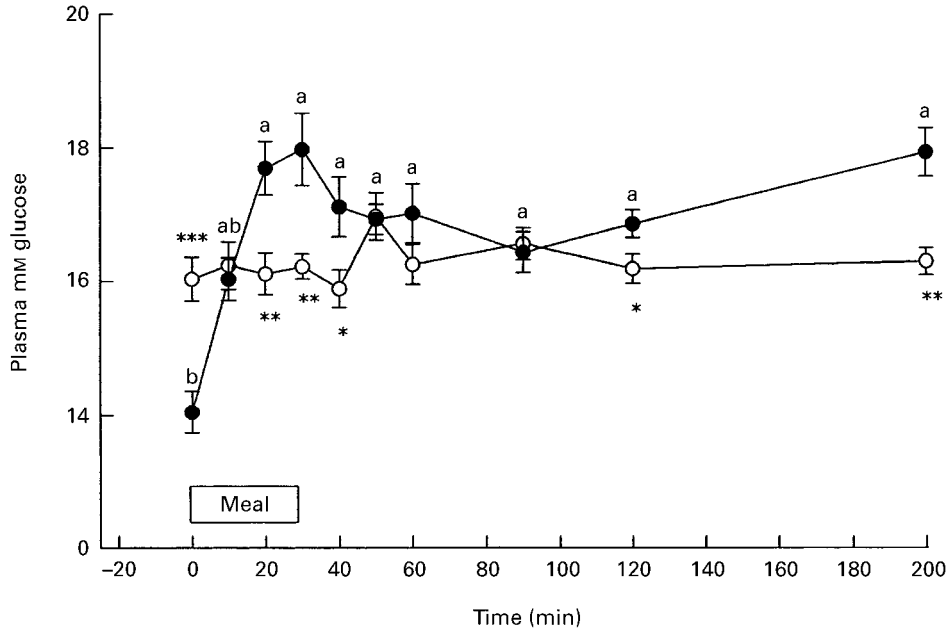
Fig. 5. Plasma glucose immediately before, during and after a 30 min meal in male broiler chickens fed *ad libitum* (○) or food-restricted (●). Values are means with their standard errors (*n* 10). For details of diets and procedures, see p. 642. [a,b]Mean values with unlike superscript letters were significantly different between the sampling times of food-restricted chickens (Scheffe test, $P<0.05$). Mean values were significantly different from food-restricted male broiler chickens within each sampling time: *$P<0.05$, **$P<0.01$, ***$P<0.001$.

plasma GH levels, being the mechanistic result of a much more pronounced pulsatile GH release as reflected in a higher mass of GH secreted per burst and shorter pulse interval. The reduced hepatic GH receptor capacity of FR chickens was also assumed to be the reason, at least in part, for the lower plasma IGF-I levels of these chickens.

This uncoupling of IGF-I from GH, which is also recognised in other animal species and in man, represents a mechanism to reduce muscle cell growth and proliferation in favour of substrate (catabolism) and energy (lipolysis) mobilisation in order to maintain homeostasis (Buyse & Decuypere, 1999). In accordance with other studies (e.g.
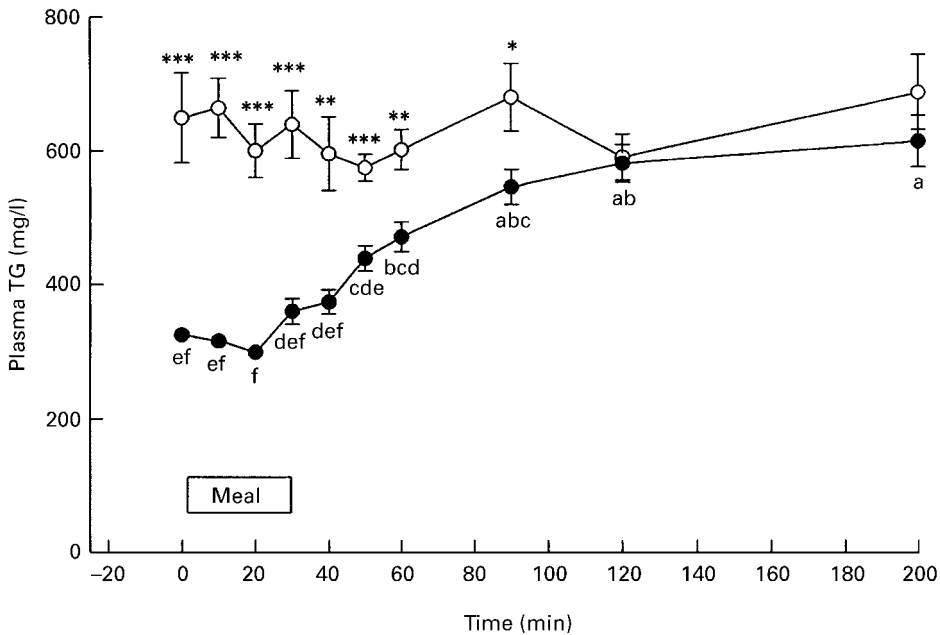

Fig. 6. Plasma triacylglycerol (TG) concentrations immediately before, during and after a 30 min meal in male broiler chickens fed *ad libitum* (○) or food-restricted (●). Values are means with their standard errors (*n* 10). For details of diets and procedures, see p. 642. [a,b,c,d,e,f]Mean values with unlike superscript letters were significantly different between the sampling times of food-restricted chickens (Scheffe test, $P<0.05$). Mean values were significantly different from food-restricted male broiler chickens within each sampling time: *$P<0.05$, **$P<0.01$, ***$P<0.001$.
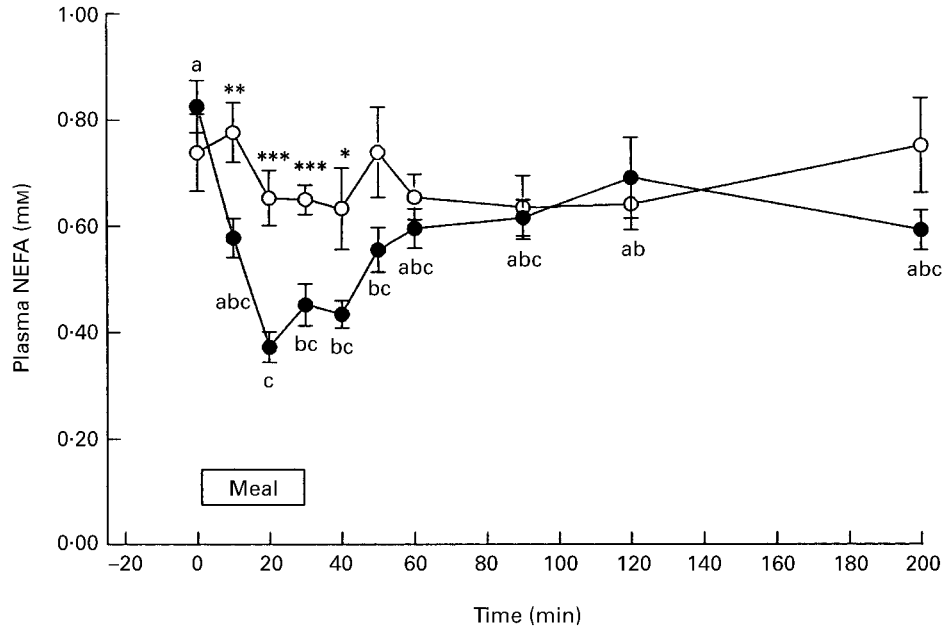
Fig. 7. Plasma non-esterified acid (NEFA) concentrations immediately before, during and after a 30 min meal in male broiler chickens fed *ad libitum* (○) or food-restricted (●). Values are means with their standard errors (*n* 10). For details of diets and procedures, see p. 642. [a,b,c]Mean values with unlike superscript letters were significantly different between the sampling times of food-restricted chickens (Scheffe test, $P < 0.05$). Mean values were significantly different from food-restricted male broiler chickens within each sampling time: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Harvey & Klandorf, 1983; Decuypere & Kühn, 1984; Van der Geyten *et al.* 1999), food deprivation was also associated with an increase and a decrease in plasma T_4 and T_3 concentrations respectively. These fasting-induced changes including the role of GH receptors in the uncoupling of IGF from GH and in the reduction in plasma T_3 levels, as well as the negative feedbacks of several peripheral humoral factors on GH secretion, have been discussed in detail elsewhere (Harvey *et al.* 1991; Buyse *et al.* 2000, 2001*a*).

Fig. 8. Plasma lactate concentrations immediately before, during and after a 30 min meal in male broiler chickens fed *ad libitum* (○) or food-restricted (●). Values are means with their standard errors (*n* 10). For details of diets and procedures, see p. 642. [a,b]Mean values with unlike superscript letters were significantly different between the sampling times of food-restricted chickens (Scheffe test, $P < 0.05$). Mean values were significantly different from food-restricted male broiler chickens within each sampling time: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.
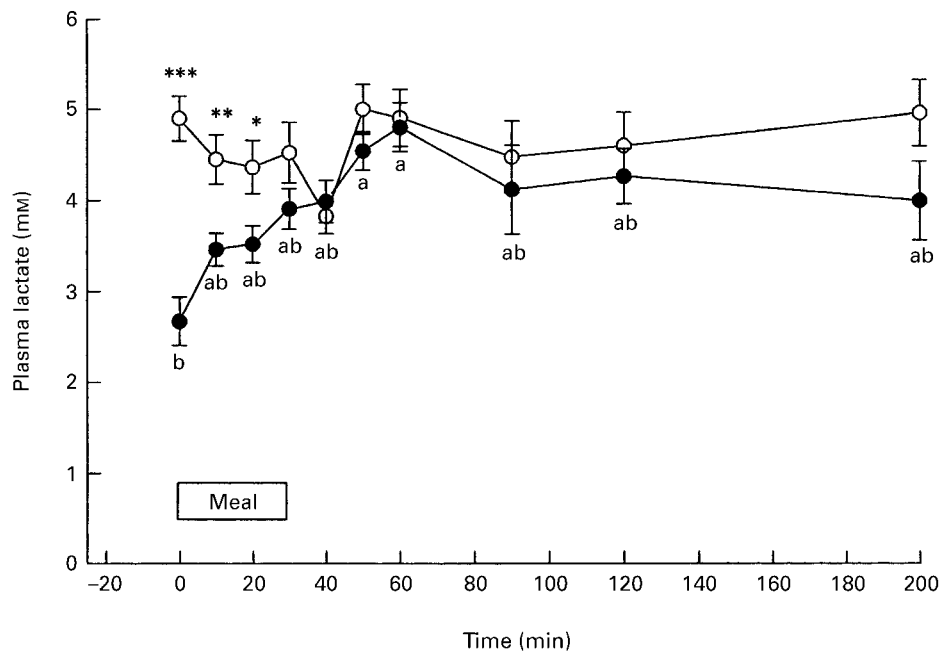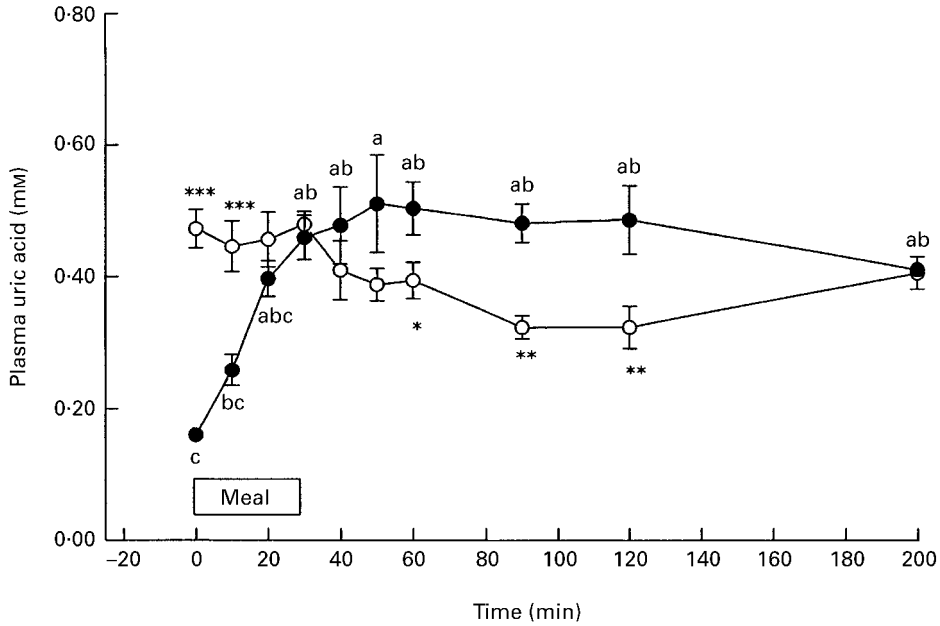
Fig. 9. Plasma uric acid concentrations immediately before, during and after a 30 min meal in male broiler chickens fed *ad libitum* (○) or food-restricted (●). Values are means with their standard errors (*n* 10). For details of diets and procedures, see p. 642. [a,b,c]Mean values with unlike superscript letters were significantly different between the sampling times of food-restricted chickens (Scheffe test, $P < 0.05$). Mean values were significantly different from food-restricted male broiler chickens within each sampling time: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

We have repeated our previous study (Buyse *et al.* 2000) and extended it with the effect of food deprivation and the postprandial time course of changes in plasma concentrations of hormones and key metabolites and hepatic D-I and -III activities during and after refeeding. In accordance with other studies in chickens (Bacon, 1986), turkeys (Anthony *et al.* 1990) and ducks (Lien *et al.* 1999), food

deprivation caused a significant decrease in plasma TG levels. The origin of TG in the circulation of avian species, *in casu* chickens, is 2-fold. Dietary fats are hydrolysed by intestinal lipases and then the fatty acids are taken up by the enterocytes. Within these cells, the fatty acids are re-esterified with glycerol to form newly constituted TG. In contrast to mammals, the lymphatic system is poorly

Fig. 10. Hepatic deiodinase (D) I activity immediately before, during and after a 30 min meal in male broiler chickens fed *ad libitum* (○) or food-restricted (●). rT$_3$, reverse triiodothyronine. Values are means with their standard errors (*n* 10). For details of diets and procedures, see p. 642. [a,b]Mean values with unlike superscript letters were significantly different between the sampling times of food-restricted chickens (Scheffe test, $P < 0.05$). Mean value was significantly different from food-restricted male broiler chickens within the sampling time: ***$P < 0.001$.
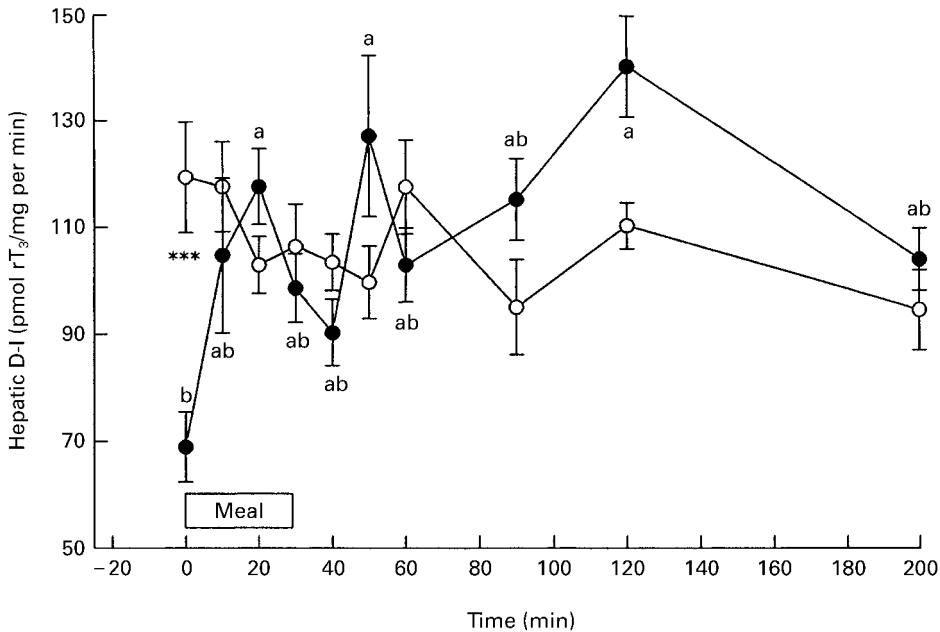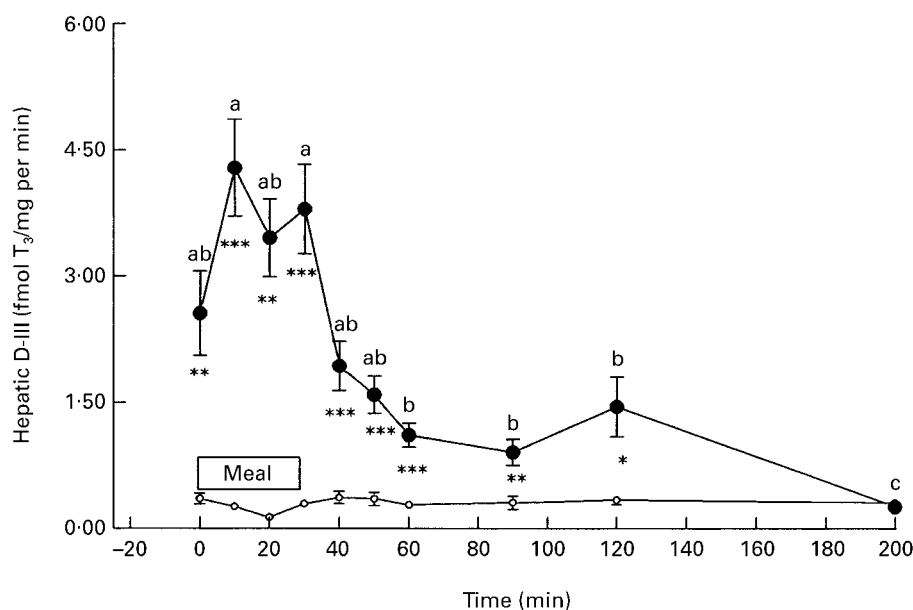
Fig. 11. Hepatic deiodinase (D) III activity immediately before, during and after a 30 min meal in male broiler chickens fed *ad libitum* (○) or food-restricted (●). T$_3$, triiodothyronine. Values are means with their standard errors (*n* 10). For details of diets and procedures, see p. 642. [a,b,c]Mean values with unlike superscript letters were significantly different between the sampling times of food-restricted chickens (Scheffe test, $P<0.05$). Mean values were significantly different from food-restricted male broiler chickens within each sampling time: *$P<0.05$, **$P<0.01$, ***$P<0.001$.

developed in avian species, so TG are transported in the form of portomicrons in the hepatic vein to the liver and further on to other peripheral tissues (Bensadoun & Rothfield, 1972). Second, plasma TG can also originate from the *de novo* lipogenesis from carbohydrates, amino acids and other non-fat precursors, a process that primarily takes place in the avian liver (Leveille *et al.* 1975). These newly synthesised TG are then transported under the form of VLDL by the blood to peripheral tissues. It is evident that both sources of TG are absent in food-deprived animals, which explains the low plasma TG levels in these animals. The *de novo* lipogenesis in the avian liver is, besides the availability of precursors, highly dependent on some key enzymes, such as malic enzyme. The activity of this enzyme is under the positive control of T$_3$, insulin and IGF-I and inhibited by glucagon (Goodridge *et al.* 1989). The strongly reduced availability of circulating T$_3$, IGF-I and insulin in FR chickens will lead to an impaired malic enzyme activity, hence to a reduced NEFA and TG synthesis. In addition, insulin is known to have lipogenic properties, whereas glucagon is antilipogenic in avian species (Simon, 1991). As fasting is associated with low circulating insulin and elevated glucagon levels in chickens (Simon, 1991), this may also be the endocrine basis of the reduced hepatic lipogenic activity in FR chickens.

The role of GH in lipogenesis in chickens is not clear (for review, see Buyse & Decuypere, 1999). Indeed, some studies revealed an increase in fat deposition in broilers treated with GH, whereas others observed the opposite. It was concluded that the pattern of GH administration (pulsatile *v.* continuous) and age of the chickens seems to determine the biological action of GH, besides its secondary effects on thyroid hormone metabolism (Vasilatos-Younken *et al.* 2000).

Most studies revealed an increase in plasma NEFA levels in FR poultry (e.g. Leveille *et al.* 1975; Bacon, 1986; Lien *et al.* 1999; Aman Yaman *et al.* 2000). In contrast, we did not observe any differences in plasma NEFA concentrations between FR and AL chickens. This, however, does not mean that there is no increased lipolytic activity in our FR chickens. The concentration of NEFA in the circulation is the result of two opposing processes: lipolysis on the one hand and increased NEFA uptake by peripheral tissues as energy source on the other hand. We hypothesise that the fasting-induced increase in lipolytic rate is counteracted by an elevated tissue uptake, resulting in a similar level of NEFA in the circulation. The presumed elevated lipolytic activity in our FR chickens is very likely to be induced by glucagon, a very potent lipolytic hormone in avian species (Langslow & Lewis, 1974) and perhaps also by GH, for which lipolytic properties have been clearly demonstrated *in vivo* (Vasilatos-Younken *et al.* 1988) and *in vitro* (Buyse *et al.* 1992, 1995). However, as far as we are aware, there are no data available on the effects of fasting on GH receptor characteristics of adipose tissue of poultry. In liver, it has been shown that GH receptors are down-regulated by high circulating GH levels as in the case of fasting (Vanderpooten *et al.* 1989; Buyse *et al.* 2000), but it remains speculative whether the same phenomenon also occurs in adipose tissue. It may well be that adipose GH receptors are not affected or even up-regulated in food-deprived animals in such a way that GH acts in concert with glucagon to promote lipolysis. Tissue-specific regulation of GH receptor mRNA expression was described in chicken brain, liver, bursa, spleen and thymus but, unfortunately, adipose tissue was not included (Hull & Harvey, 1998). This issue is currently under investigation. The presumed elevated uptake of NEFA by, e.g. the liver, could also be

a mechanism for the fasting-induced impaired lipogenesis as reflected in the strongly reduced plasma TG levels. Indeed, Leveille *et al.* (1975) suggested that CoA-activated fatty acids inhibit the hepatic fatty acid synthesis by affecting acetyl-CoA carboxylase and the citrate cleavage reaction for which free CoA is required. By using cultured chicken hepatocytes, Lien *et al.* (2000) provided direct evidence that certain NEFA, albeit with a different potency, inhibit the lipogenic activity.

Animals respond to the absence of food by behavioural and physiological mechanisms. The latter mechanisms include a reduction in metabolic rate, an increase in lipolysis and ultimately in protein catabolism. A reduction in metabolic rate or heat production of fasting chickens has been demonstrated together with a concomitant decrease in plasma T_3 levels (Klandorf *et al.* 1981). Hence, our present observations that plasma T_3 levels are significantly ($P < 0.001$) reduced by food deprivation provide indirect evidence that our FR chickens have also reduced their metabolic rate. Plasma lactate levels were significantly depressed in FR chickens compared with the levels of their AL counterparts. In view of the reduced metabolic rate of FR animals, there is no need for anaerobic energy production, hence lactate production from glucose is limited. Plasma lactate levels are indeed positively correlated with metabolic rate as observed during the period of highest growth and hence metabolic rate (Chineme *et al.* 1995). It can also be inferred that any lactate produced is transported directly to the liver for immediate gluconeogenesis in fasted animals (Cori cycle), leading to low plasma lactate levels.

In birds, purine bases are degraded to uric acid. In addition, purines are formed from excess amino-N, which are subsequently degraded to uric acid and excreted in the urine (Stryer, 1981). Fasting significantly impairs fractional and absolute protein synthesis rate in chickens (Aman Yaman *et al.* 2000), but this is not necessarily associated with concomitant increases in protein catabolism, as indicated by the low uric acid levels of our 23·5 h FR chickens. It is hypothesised that protein breakdown only occurs after prolonged fasting. It is also too simplistic a view to believe that a starved animal will firstly mobilise all of its fat reserves before protein catabolism is initiated. Indeed, we have previously observed that fasting laying hens for 3 d is associated with significantly reduced plasma uric acid levels and a high lipolytic activity (Buyse *et al.* 1995). However, after a prolonged period of 7 d of fasting, plasma uric acid levels were increased again, resulting from the catabolism of endogenous proteins, despite the presence of still considerable amounts of body fat stores.

Refeeding

The postprandial changes in plasma hormone levels are in accordance with our previous study (Buyse *et al.* 2000). Mean plasma GH levels decreased readily after food provision, which is the consequence of a reduction in amplitude, mass secreted per burst, and an increase in pulse interval (Buyse *et al.* 2000). The mechanisms involved in the altered pituitary GH secretion have been discussed in detail elsewhere (Buyse *et al.* 2000, 2001a). Plasma

IGF-I levels rose gradually after refeeding but did not reach AL values after 200 min. Kita *et al.* (1998b) did observe a normalisation of circulating IGF-I levels after 2 h of refeeding in leghorn chickens that were fasted for 2 d but, surprisingly, plasma IGF-I dropped again to fasting levels after 6 h of refeeding. A very similar time pattern was observed for hepatic IGF-I mRNA levels. In a subsequent study, Kita *et al.* (1998a) again observed that hepatic IGF-I mRNA increased to AL levels after 120 min of refeeding, but only when the diet consisted of protein and carbohydrate and not of fat. Differences in experimental design between our experiments and those of Kita *et al.*, such as strain and age of chickens, fasting duration and a single or repeated periods of fasting–refeeding, may account for the differences in the time course of changes of IGF-I levels as well as of other physiological processes such as lipogenesis (Leveille *et al.* 1975). It may well be that under our present experimental conditions, plasma IGF-I levels never reach values similar to those of their AL counterparts.

In accordance with our previous study (Buyse *et al.* 2000), plasma T_4 levels of FR chickens remained significantly ($P < 0.001$) higher during the entire sampling session and only decreased slowly after about 90 min. Plasma T_3 levels gradually increased postprandially, although at a slower rate than in the first experiment (Buyse *et al.* 2000). In the present experiment, we observed a significant decrease in hepatic D-I activity of FR chickens, which is in contrast with our previous study (Buyse *et al.* 2000) and other experiments (Van der Geyten *et al.* 1999) reporting no effect of fasting on hepatic D-I activity. However, all experiments clearly showed a marked increase in hepatic D-III activity in fasting animals, followed by a gradual normalisation after the introduction of food, although in the present study, somewhat slower than observed previously. It is believed that D-III is much more susceptible than D-I to nutritional manipulations such as fasting and refeeding or diet composition (Buyse *et al.* 2001a). As Van der Geyten *et al.* (1999) has already pointed out, hepatic as well as renal D-III activities are strongly involved in the regulation of plasma T_3 levels.

Metabolite–endocrine interactions

It is obvious from our present results that the postprandial changes in plasma metabolite levels proceed more quickly than those of hormones, and even between metabolites there are significant differences in the time needed to attain statistically significant changes compared with the fasting levels or to reach the levels of AL counterparts. The order of responsiveness after food availability is broadly: glucose > uric acid ≥ NEFA > lactate > TG. For the plasma hormones, the order seems to be: GH > T_3 > T_4 > IGF–I.

It is tempting to make functional relationships between these humoral variables based on their differential time courses in postprandial changes. Glucose seems to be a primary trigger of these postprandial alterations. First, glucose is known to exert a negative feedback on GH secretion (Harvey *et al.* 1991) and may act in concert with other hormones such as insulin in order to induce the postprandial

decrease in plasma GH levels. Second, fasting is associated with reduced hepatic GH receptor capacity and this may be partially caused by the high GH levels, which down-regulate their own hepatic receptors (Vanderpooten *et al.* 1989), but also by the low glucose levels. Indeed, it has been shown *in vitro* by using rat (Niimi *et al.* 1991) or porcine (Brameld *et al.* 1999) hepatocytes that glucose is strongly involved in the induction of GH receptor, and this might also be the case in avian species. Third, glucose is known to stimulate hepatic D-I activity (Gavin & Moeller, 1983; Klandorf & Harvey, 1985). We have indeed observed a sharp augmentation in hepatic D-I activity within 10 min after meal initiation in parallel with the increase in plasma glucose levels. Based on these considerations, it is hypothesised that a major post-prandial function of glucose is to restore hepatic GH receptor capacity. This may then allow GH to exert some of its biological actions such as T_3 and IGF-I production. However, we were previously not able to discern a postprandial increase in hepatic GH receptor capacity (Buyse *et al.* 2000), but it must be recognised that the specific hepatic GH binding in FR and early refed chickens was very low and small but, nevertheless, physiologically meaningful changes might not have been picked up by this ligand-binding assay. By using the competitive reverse transcrip-tase-polymerase chain reaction technique for GH receptors (Van As *et al.* 2001), we were able to quantify the extra-cellular and intracellular mRNA concentrations in total liver mRNA of chickens. The extracellular and intra-cellular GH receptor mRNA concentrations were signifi-cantly ($P<0.05$) reduced in the FR chickens compared with the control chickens. Refeeding induced the expression of both GH receptor mRNA, but the level of expression never reached the level of that of AL chickens. The concentration of GH receptor mRNA even seemed to decline at 200 min after meal initiation (P Van As, unpub-lished results). Fourth, hexoses may also directly affect lipogenesis and lipolysis as intravenous administration of a glucose or fructose solution to fasted chickens increased *in vitro* hepatic lipogenesis and decreased plasma NEFA within 45 min, in a similar way as refeeding a complete meal (Leveille *et al.* 1975). Our present results are also in accordance with the findings of Leveille *et al.* (1975) that the restoration of lipogenesis after refeeding is pre-ceded by a reduction in circulating NEFA levels.

Although both T_3 and IGF-I are mainly produced in the liver and the fact that their production is highly dependent on GH, the increase in plasma IGF-I levels lagged behind that of T_3. This might be related to their different generat-ing mechanisms, as transcription and translation of the IGF gene might require more time than D enzyme (in)activ-ation. Kita *et al.* (1998*a*) indeed noticed only a significant increase in hepatic IGF-I mRNA levels after 120 min of refeeding, whereas we found that D-I and -III activities were already significantly altered after 10 and 60 min respectively. Furthermore, Tsukada *et al.* (1998) clearly demonstrated that thyroid hormones are needed for stimu-lating hepatic GH receptor gene expression and hence are required for the GH-dependent IGF-I production. It seems therefore conceivable that plasma T_3 levels increase before that of IGF-I after refeeding.

The initial postprandial decrease in plasma NEFA levels is likely to be the result of a reduction in lipolytic activity. Surprisingly, plasma NEFA then increased again to values of their AL counterparts. It is speculated that the uptake of NEFA in peripheral tissues is strongly reduced due to the availability of carbohydrates as primary energy source. Subsquently, the hepatic lipogenesis is promoted as the consequence of increased availability of free CoA, leading to an increase in circulating TG levels. There is no doubt that the initiation of food intake is immediately followed by a pronounced increase in heat production due to physi-cal activity and dietary-induced thermogenesis. Further-more, the fractional rate of breast muscle protein synthesis, an energetically costly process, is increased sig-nificantly within 60 min after a 2 d fasting period (Aman Yaman *et al.* 2000). In view of the fact that plasma lactate levels increased sharply after meal initiation, and even pre-ceded by far the response in plasma T_3 levels, it can be argued that the refed birds initially rely heavily on anaero-bic pathways to sustain this burst of energy requirements. It was unexpected to observe such a rapid postprandial increase in plasma uric acid levels but this could be the consequence of oxidation of amino acids for energy purposes.

In conclusion, subjecting young meat-type chickens to a single daily meal compromises their genetic potential for fast growth to a great extent. This growth retardation during the rearing period is needed in order to ensure an acceptable adult reproductive performance. This manage-ment practice forces the chickens to change their metabol-ism from anabolism (growth) to mechanisms aimed at maintaining body homeostasis. Indeed, metabolic rate is reduced (low plasma T_3 levels) due to changes in GH-receptor-dependent hepatic D activities in order to spare bodily reserves. This reduced metabolism is also reflected in low plasma lactate levels. Although protein synthesis will be reduced by food deprivation, the breakdown of body protein is not yet initiated after about 24 h of fasting. Finally, food deprivation of chickens is associated with a shift from lipogenesis towards increased fatty acid turnover and an uncoupling of IGF-I from GH. These alterations in intermediary metabolism are governed by endocrine factors.

Refeeding reverses these processes after concomitant opposite changes in endocrine functioning. However, the postprandial time courses of changes in circulating metab-olites precedes those of hormones. The relationship between the (neuro)endocrine system and the intermediary metabolism is not a 'one-direction' phenomenon as both systems undoubtedly interact with each other. Glucose is believed to be the primary trigger for the postprandial changes in metabolic and endocrine functioning. In addition, the hepatic GH receptor seems to play a central role in these meal-induced changes.

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