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Leucine Degradation In Chick Skeletal Muscle

by

Wu Guoyao

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Léucine Degradation In Chick Skeletal Muscle submitted by Wu Guoyao in partial fulfilment of the requirements for the degree of Master of SCIENCE in Animal Biochemistry.

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Abstract

— The effects of fasting, ketone bodies, octanoate, glucose and prostaglandins E_2 and $F_2\alpha$ on leucine degradation in 10-day-old chick *extensor digitorum communis* muscles were investigated *in vitro*. The net rate of leucine transamination and the rates of leucine oxidative decarboxylation, oxidation of leucine carbons 2-6 and CO_2 production from total leucine oxidation were 0.567 ± 0.029, 0.415 ± 0.021, 0.952 ± 0.075 and 1.366 ± 0.092 nmol^{-1} mg muscle^{-1} for fed chicks, respectively. Fasting for 24 h increased ($P<0.01$) these rates above those obtained with 12-h fasted and fed groups. In muscles from fed chicks, 4 mM DL-β-hydroxybutyrate increased ($P<0.01$) the rate of net leucine transamination; both 4 mM DL-β-hydroxybutyrate and 1 or 4 mM acetoacetate inhibited ($P<0.05$) the rate of leucine oxidative decarboxylation and increased ($P<0.01$) the net rate of α-ketoisocaproate (KIC) production and the percentage of transaminated leucine released as KIC. In muscles from 24-h fasted chicks, 4 mM DL-β-hydroxybutyrate and 1 or 4 mM acetoacetate inhibited ($P<0.01$) the rates of net leucine transamination and leucine oxidative decarboxylation. The inhibtion of both leucine oxidative decarboxylation and total leucine oxidation by ketone bodies is independent of leucine uptake, and insulin, glucose and amino acid concentrations. Octanoate at 0.2 and 1 mM markedly increased ($P<0.01$) the net rate of leucine transamination and the rates of leucine oxidative decarboxylation, oxidation of leucine carbons 2-6 and CO_2 production from total leucine oxidation in muscles from fed chicks, but had no effect on muscles from fasted chicks. Five mM and 12 mM glucose decreased ($P<0.01$) the rates of leucine oxidative decarboxylation, oxidation of leucine carbons 2-6 and CO_2 production from total leucine oxidation and increased ($P<0.05$) the net rate of KIC production as compared to the control (no glucose) group in muscles from fed chicks, but had no effect in muscles from fasted birds. Arachidonic acid (5 μM), prostaglandin E_2 (0.28 μM) and $F_2\alpha$ (14 μM) inhibited the net rate of leucine transamination ($P<0.01$) and the rates of leucine oxidative decarboxylation ($P<0.05$), oxidation of leucine carbons 2-6 ($P<0.05$) and CO_2 production from total leucine oxidation ($P<0.01$) in muscles from fed chicks. Indomethacin at 5 and 50

μM markedly inhibited ($P<0.01$) the rate of PGE_2 production by skeletal muscle in the presence of exogenous arachidonic acid and prevented the inhibition of arachidonic acid on leucine degradation. These results demonstrate that (1) fasting increases the rate of leucine degradation in chick skeletal muscle; (2) ketone bodies inhibit the rate of leucine degradation in muscles from fed and fasted chicks; (3) octanoate and glucose regulate leucine degradation in skeletal muscle depending on initial activity of BCKA dehydrogenase and (4) prostaglandins may play a role in the regulation of leucine degradation in skeletal muscle.

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I. Introduction

The skeletal musculature is normally the largest tissue in animals and accounts for approximately 25% and 45% of body weight in the newborn and young adult, respectively (Goll et al., 1977). Traditionally, the skeletal musculature was considered to be a relativey inert protein reservoir whose constituent amino acids are mobilized under conditions such as fasting and disease. This view, however, has changed since Johnson et al. in 1961 first reported that $^{14}CO_2$ is produced from L-[1-^{14}C]leucine by the rat diaphragm. It has become well established that skeletal muscle extensively metabolizes the branched-chain amino acids (BCAA); leucine, isoleucine and valine as well as alanine, glutamic acid, glutamine, aspartic acid and asparagine (Goldberg and Chang, 1978; Davis and Lee, 1985). In Chapter 1 of this thesis, the physiological significance of leucine and the regulation of leucine metabolism in skeletal muscle will be briefly reviewed. A number of reviews of these topics have been published in the last six years (e.g., Snell, 1980; Harper et al., 1983; 1984; Randle et al., 1984).

A. Physiological Significance of Leucine Metabolism

Leucine has been shown to stimulate protein synthesis and to inhibit protein degradation in rat skeletal muscle under catabolic states such as *in vitro* incubation and fasting (Tischler et al., 1982; Mitch and Clark, 1984). Reports of the anabolic effects of leucine in skeletal muscle have prompted the clinical use of leucine in the improvement of nitrogen balance during catabolic states (Freund et al., 1983) and have led some researchers (e.g. Boebel and Baker, 1982) to suggest that leucine analogs α-ketoisocaproate (KIC) and D-α-hydroxyisocaproate could be included in chick and rat diets to spare this essential amino acid and decrease the burden on the liver to convert toxic ammonia to nontoxic products. A small dietary excess of leucine has been shown to increase the protein synthetic potential of porcine skeletal muscle *in vitro* (Smith, 1985). In addition, leucine can inhibit the oxidation of pyruvate and glucose (Chang and Goldberg, 1978) and increase the synthesis of alanine and

glutamine (Goldberg and Chang, 1978; Caldecourt et al. 1985) in incubated muscles. Transamination of leucine to form KIC is required for inhibition of protein degradation and for inhibition of glucose and pyruvate oxidation in skeletal muscle (Tischler et al., 1982; Mitch and Clark, 1984). In addition, since leucine is not synthesized by animals, the rate of leucine degradation in skeletal muscle can influence the dietary requirement of this amino acid as well as that of isoleucine and valine which are antagonistic to leucine (Harper et al., 1983). Furthermore, the concentration of leucine and the rate of leucine degradation in skeletal muscle can influence its concentration in plasma (Adibi, 1971; Hutson and Harper, 1981), which in turn regulates insulin release by β-cells in the pancreas (Milner, 1970) and consequently plasma insulin levels (Meguid et al., 1986). Thus, studies of leucine degradation help enhance our understanding of its role in the control of protein deposition in skeletal muscle, food intake, amino acid antagonism, interorgan metabolism of nitrogen and carbon, operation of the glucose-alanine cycle and overall energy homeostasis of an organism.

B. Leucine Metabolism

1. General Pathway

Leucine, insofar as is known, has two fates in skeletal muscle. It either serves as a precursor for tissue protein synthesis or is catabolized to provide acetyl-CoA and energy. A schematic outline of the metabolism of leucine is shown in Fig. I-1. The initial step in the catabolism of leucine is reversible transamination to KIC by BCAA aminotransferase (EC 2.6.1.42). The KIC may be released from the myofiber to the extracellular space or undergo irreversible oxidative decarboxylation to produce isovaleryl-CoA by branched-chain α-ketoacid (BCKA) dehydrogenase (EC 1.2.4.4). Dehydrogenation of isovaleryl-CoA by isovaleryl-CoA dehydrogenase results in the production of β-methylcrotonyl-CoA which is subsequently converted to acetoacetate and acetyl-CoA. Thus, leucine is a ketogenic amino acid. Skeletal muscle has been shown to release relatively large amounts of isovaleric acid (Spydevold and

Hokland, 1983) suggesting that considerable amounts of isovaleryl-CoA may be converted to isovaleric acid in this tissue rather than entering the tricarboxylic acid cycle as acetyl-CoA. The oxidation of leucine may supply an appreciable amount of energy for muscle metabolism.

2. Properties and Activity of BCAA Aminotransferase

Three isoenzymes of BCAA aminotransferase have been identified and characterised in different rat tissues (Ichihara et al., 1975; Kadowaki and Knox, 1982). Isoenzyme I accepts all three branched-chain amino acids as substrates (Harper et al., 1984). This isoenzyme is almost equally distributed between the cytosol and mitochondria and is the only form of the enzyme at these two sites in skeletal muscle cells (Kadowaki and Knox, 1982). The Km values for leucine in rat skeletal muscle range from 0.4 mM to 0.5 mM (Odessey and Goldberg, 1979; Kadowaki and Knox, 1982), which fall within the physiological levels of leucine in plasma and skeletal muscle (Adibi, 1971; Livesey and Lund, 1980). The major amino group acceptor is α-ketoglutarate for which the Km values range from 0.1 to 0.2 mM in rat skeletal muscle (Odessey and Goldberg, 1979). Very little activity is observed with pyruvate and none with oxaloacetate (Harper et al., 1984). Isoenzyme II is present in the cytosol of the liver, but has not been found in skeletal muscle (Ichihara et al., 1975; Kadowaki and Knox, 1982); while isoenzyme III has only been reported to be present in small amounts in the cytosol of skeletal muscle from 5-day-old rats (Kadowaki and Knox, 1982).

The activity of BCAA aminotransferase in skeletal muscle is much higher than that in the liver but lower than that in the heart and the kidney (Featherston and Horn, 1973; Shinnick and Harper, 1976). However, based on the relative mass of the skeletal musculature, this tissue has been generally accepted to be the main site for the transamination of leucine in the body (Shinnick and Harper, 1976).

3. Properties and Activities of BCKA Dehydrogenase

Like pyruvate dehydrogenase, BCKA dehydrogenase is a multienzyme complex (Randle et al., 1984). It is composed of three separate catalytic subunits held together by noncovalent interaction: (1) BCKA decarboxylase (E_1) arranged in an $\alpha_2\beta_2$ substructure with thiamin pyrophosphate as a prosthetic group; (2) dihydrolipoyl transacylase (E_2) with lipoate as a prosthetic group; and (3) dihydrolipoyl dehydrogenase (E_3) with FAD^+ as a prosthetic group. In addition to these covalently bound prosthetic groups, the oxidation of BCKA also requires coenzyme A (CoA-SH), NAD^+ and Mg^{2+} as cofactors. Unlike BCAA aminotransferase, BCKA dehydrogenase is exclusively located on the inner surface of the inner mitochondrial membrane (Van Hinsbergh et al., 1979). This enzyme complex catalyzes the oxidative decarboxylation of the three branched-chain α-ketoacids- KIC, α-ketomethylvalerate and α-ketoisovalerate from leucine, isoleucine and valine, respectively, to form their corresponding decarboxylated acyl-CoA derivatives. The Km value for KIC in skeletal muscle has been reported to be 25 μM (Odessey and Goldberg, 1979), which falls within the physiological range of concentrations of KIC in plasma (Hutson and Harper, 1981).

The activity of BCKA dehydrogenase is relatively low in skeletal muscle (Featherston and Horn, 1973; Shinnick and Harper, 1976; Patston et al., 1984; Wagenmakers et al., 1984a). The total activity and actual activity of BCKA dehydrogenase in fed rat skeletal muscle have been reported to be 30 and 1.6 nmol/min/g tissue, respectively (Wagenmakers et al., 1984a). Changes in the activity of this enzyme play a key role in the regulation of BCAA degradation by skeletal muscle as studies with cell-free extracts clearly indicate that oxidative decarboxylation of BCKA is the rate-limiting step for their catabolism in this tissue (Odessey and Goldberg, 1979).

C. Regulation of Leucine Degradation in Skeletal Muscle

1. Phosphorylation and Dephosphorylation of BCKA Dehydrogenase

Observations that either the depletion of ATP by preincubation or the addition of a broad-spectrum phosphatase activates BCKA dehydrogenase while the addition of ATP inactivates this enzyme in muscle mitochondria (Odessey , 1980; Parker and Randle, 1980; Fatania et al., 1982; Paul and Adibi, 1982) have led to the proposal that the BCKA dehydrogenase complex is subject to regulation by ATP-dependent phosphorylation and dephosphorylation (Randle et al., 1984) like the pyruvate dehydrogenase. Direct evidence has been recently shown by Paxton et al. (1986) that there is an inverse linear relationship between the extent of phosphorylation of serine hydroxyl groups of BCKA dehydrogenase and the activity of this enzyme in skeletal muscle. BCKA dehydrogenase kinase, which catalyses the phosphorylation of BCKA dehydrogenase, and BCKA dehydrogenase phosphatase, which catalyses the dephosphorylation of BCKA dehydrogenase, have been characterised (Paxton and Harris, 1984; Damuni et al., 1984). Many factors have been reported to regulate the rate of leucine metabolism in skeletal muscle largely through their action on BCKA dehydrogenase (Paxton and Harris, 1984).

2. Effect of Oxidative Substrates

The effects of fatty acids, ketone bodies and glucose on leucine degradation in skeletal muscle have been extensively studied in the rat. Variable results have been reported possibly due, in part, to differences in the experimental conditions under which the studies were performed. Buse et al. (1972) first reported that long chain fatty acids such as palmitate and oleate markedly stimulate both the oxidative decarboxylation and total oxidation of BCAA in incubated hemidiaphragms from fed rats. However, the stimulating effect of long chain fatty acids was not confirmed by Paul and Adibi (1976) using skeletal muscle homogenates. In isolated intact skeletal muscle (Buse et al., 1972; 1975; Wagenmakers and Veerkamp, 1984b),

perfused hindquarter (Spydevold and Hokland, 1981) and skeletal muscle homogenates (Paul and Adibi, 1976), octanoate has been shown to increase leucine oxidative decarboxylation probably by inhibiting BCKA dehydrogenase kinase (Paxton and Harris, 1984). Whether octanoate stimulates leucine decarboxylation in skeletal muscle from fasted rats remains controversial (Paul and Adibi, 1976; Wagenmakers and Veerkamp, 1984b).

Variable effects of glucose on leucine decarboxylation in skeletal muscle have been reported. For example, glucose has been shown to inhibit the rate of leucine oxidative decarboxylation in hemidiaphragms from fed rats (Buse et al., 1972; Odessey and Goldberg, 1972). However, the addition of glucose to incubation media has not been reported to influence the rate of leucine decarboxylation in the intact rat hemidiaphragm (Wagenmakers et al., 1984b) and skeletal muscle homogenates (Paul and Adibi, 1976). Thus, it is not clear whether changes in concentrations of glucose affect leucine catabolism in skeletal muscle *in vivo*.

Ketone bodies are extensively metabolized by skeletal muscle as oxidative substrates with acetoacetate being utilized to a greater extent than β-hydroxybutyrate (Ruderman and Goodman, 1973). Thus, the oxidation of ketone bodies may spare leucine as an oxidative substrate. Buse et al. (1972) first reported that DL-β-hydroxybutyrate (4 mM) inhibited the rate of oxidative decarboxylation of leucine in hemidiaphragms from fed rats. Wagenmakers and Veerkamp (1984c) have recently shown that DL-β-hydroxybutyrate (4 mM) plus acetoacetate (1 mM) decreases the oxidative decarboxylation of BCAA and BCKA in rat skeletal muscle. In contrast, Paul and Adibi (1978) have demonstrated that acetoacetate (2-20 mM) stimulates the rate of leucine oxidative decarboxylation in skeletal muscle homogenates from both fed and fasted rats and that DL-β-hydroxybutyrate has no effect at concentrations less than 30 mM which are well above physiological concentrations in plasma. Thus, it appears that the intact skeletal muscle and skeletal muscle homogenates respond to ketone bodies in an opposite manner.

3. Availability of Amino Group Acceptors and BCKA Dehydrogenase Cofactors

The availability of amino group acceptors may also limit the transamination rate of leucine and thus the flux of KIC through BCKA dehydrogenase. For example, the addition of α-ketoglutarate has been shown to increase the oxidative decarboxylation of leucine in skeletal muscle homogenates (Paul and Adibi, 1976; Van Hinsbergh et al., 1979) and in the intact hemidiaphragm from the rat (Buse et al., 1975) probably by increasing the rate of transamination of leucine. It has been shown recently that pyruvate stimulates the transamination of BCAA in the rat diaphragm (Aftring et al., 1985) likely by increasing the rate of glutamate transamination and providing more α-ketoglutarate for leucine transamination.

The oxidative decarboxylation of BCKA requires NAD^+ and CoA-SH as cofactors (Randle et al., 1984). Low concentrations of these cofactors may limit the decarboxylation of BCKA and thus the total oxidation of BCAA since decarboxylation is the rate-limiting step for catabolism of BCAA in skeletal muscle (Odessey and Goldberg, 1979). A deficiency of both of these cofactors may occur in muscles during conditions such as the oxidation of relatively large amounts of fatty acids and ketone bodies. In addition, NADH has been reported to be a potent competitive inhibitor of the BCKA dehydrogenase complex (Randle et al., 1984). The ratio of NADH to NAD^+ has been suggested to control the flux of BCKA through BCKA dehydrogenase in skeletal muscle from diabetic rats (Buse et al., 1976).

4. BCAA and Their Metabolites

The establishment of high concentrations of leucine increases its rate of transamination and decarboxylation in isolated skeletal muscle preparations (Buse et al., 1975; Van Hinsbergh et al., 1979; Mitch and Clark, 1984). Recently, it has been shown by Aftring et al. (1986) that leucine and isoleucine injected into the rat to achieve physiological concentrations in the circulatory system activates BCKA dehydrogenase subsequently isolated from skeletal muscle. The addition of KIC to incubation media has also been shown to

increase KIC decarboxylation in skeletal muscle (Van Hinsbergh et al., 1979) by stimulating the activity of BCKA dehydrogenase through inhibiting BCKA dehydrogenase kinase (Paxton and Harris, 1984). Isovaleryl-CoA, the product of the oxidative decarboxylation of KIC, is a potent inhibitor of BCKA dehydrogenase (Randle et al., 1984). The accumulation of this acyl-CoA may be regulated by the isovaleryl-CoA dehydrogenase which has rather low activity in skeletal muscle (Rhead et al., 1982). Thus isovaleryl-CoA dehydrogenase may play a role in controlling the oxidative decarboxylation and the oxidation of carbons 2-6 of the decarboxylated leucine in skeletal muscle.

5. Hormones

Regulation of BCAA aminotransferase activity by hormones has not received much attention. A small rise in the activity of this enzyme in skeletal muscle was reported in cortisol-treated rats (Mimura et al., 1968). The addition of insulin has been shown by Hutson et al. (1978; 1980) to decrease the transamination of leucine in the perfused hindquarter of fed rats. Since leucine transamination was not measured in most studies involving hormones, it cannot be said with certainty that hormone-induced changes in total oxidation of BCAA are due to changes in leucine oxidative decarboxylation alone as regulation may occur at the transamination step as well (Aftring et al., 1985).

Early studies showed that the addition of insulin increases the oxidative decarboxylation of BCAA in hemidiaphragms from fed rats (Manchester, 1965). To the contrary, it has more recently been shown that insulin decreased the rate of leucine decarboxylation in the perfused hindquaters from fed rats (Hutson et al., 1978; 1980) but stimulated this reaction in the perfused hindquaters of fasted rats (Hutson et al., 1980). In skeletal muscle homogenates, however, insulin has been reported not to influence leucine decarboxylation (Paul and Adibi, 1976). Epinephrine has been shown to increase BCAA oxidation by diaphragms from starved rats (Buse et al., 1973) and to have no effect in diaphragms (Buse et al., 1973) and skeletal muscle homogenates from fed rats (Paul and

Adibi, 1976). Although glucagon has been shown to increase the rate of BCAA catabolism in the perfused heart, it has not been shown to affect oxidation of BCAA by rat diaphragm (Buse et al., 1973) and skeletal muscle homogenates (Paul and Adibi, 1976).

6. Short-Term Fasting

The effect of fasting on leucine degradation in skeletal muscle of rats has been extensively studied. Goldberg and Odessey (1972) first reported that fasting for 2-3 days markedly increased the rate of oxidative decarboxylation of leucine in rat hemidiaphragms. These early findings have been confirmed by subsequent studies in both intact skeletal muscle and skeletal muscle homogenates (Paul and Adibi, 1976; Odessey and Goldberg, 1979; Wagenmakers and Veerkamp, 1984a; Aftring et al., 1985).

The increased leucine degradation during fasting is independent of leucine uptake by skeletal muscle (Nallathambi et al., 1972). In addition, the increased leucine oxidation is not simply a result of isotope dilution since Aftring et al. (1985) have shown that there is no difference in the intracellular specific activity of leucine in skeletal muscles from fed rats and from fasted rats (Aftring et al., 1985). Increases in activities of both BCAA aminotransferase (Adibi et al., 1975) and BCKA dehydrogenase during fasting (Odessey and Goldberg, 1979) have been shown to result in increased leucine degradation in skeletal muscle.

Although it is well documented that fasting increases the oxidation of leucine in rat skeletal muscle, Wijayasinghe et al. (1983) have shown that leucine degradation is decreased in intact intercostal myofiber bundle preparations from 5-day fasted sheep. These findings suggest that the response of BCAA metabolism in skeletal muscle to fasting may be species-dependent.

D. Leucine Degradation in Avian Species

Although the activity of BCAA aminotransferase and BCKA dehydrogenase in skeletal muscle homogenates from domestic chicks (Featherston and Horn, 1973) and Japanese quail (Mason and Ward, 1979) have been reported, there is little information available concerning leucine degradation and its regulation by oxidative substrates, hormones and food deprivation in skeletal muscle from avian species. These early enzymatic assays with muscle homogenates, carried out in the absence of inhibitors of BCKA dehydrogenase kinase and phosphatase, may be misleading since it is well established that BCKA dehydrogenase is regulated by phosphorylation and dephosphorylation (Randle et al., 1984). Therefore, the reported lack of changes in the activity of BCKA dehydrogenase in skeletal muscle homogenates from chicks fed protein-free diets, high-protein diets or fasted for 48 h do not neccessarily demonstrate that leucine catabolism in chick skeletal muscle is unaltered under these conditions.

E. Prostaglandins

Prostaglandins (PG) are cyclopentane derivatives formed from polyunsaturated fatty acids (e.g., arachidonic acid) and released by most animal cell types in response to a variety of physiological and pathological changes (Samuelsson et al., 1978; Needleman et al., 1986). PGE_2, $PGF_2\alpha$, PGD_2, 6-keto-$PGF_1\alpha$ and thromboxane B_2 have been shown to be produced by both human and rat skeletal muscle (Rodemann and Goldberg, 1982; Freund et al., 1985; Berlin et al., 1979; Nowak et al., 1983). The binding of prostaglandins to their receptors on the plasma membrane of target cells produces second messengers, resulting in alterations of a number of different biochemical events (Robertson, 1986). For example, $PGF_2\alpha$ has been shown to stimulate phosphorylation of 40S ribosomal protein S6 in Swiss Mouse 3T3 cells (Thomas et al., 1982). $PGF_2\alpha$ has also been reported to increase the rate of protein synthesis in skeletal muscle *in vitro* (Rodemann and Goldberg, 1982; Smith et al., 1983), and mediate the stimulating effect of insulin on protein synthesis in skeletal muscle *in vitro* (Reeds and Palmer, 1983) and *in vivo* (Reeds et al., 1985). On the other hand, PGE_2 has been shown to

stimulate protein degradation in rat skeletal muscle *in vitro* and mediate the stimulating effect of interleukin-1 on protein degradation in rat skeletal muscle (Baracos et al., 1983). PGE_1 and PGE_2 have also been shown to enhance hepatic gluconeogenesis (Sacca et al., 1974) and glucose oxidation in rat adipocytes (Chang and Roth, 1981). Despite the reported effects of prostaglandins on protein turnover and glycolysis in skeletal muscle (Rodemann and Goldberg, 1982; Leighton et al., 1985), there have been no reports in the literature concerning the effects of these compounds on individual amino acid metabolism in skeletal muscle.

F. Objectives of The Present Study

This literature review indicates that BCKA dehydrogenase and BCAA aminotransferase in rat skeletal muscle are subject to regulation by availability of cofactors such as NAD^+ and CoA-SH, NADH, oxidative substrates, metabolites of BCAA, hormones, and ATP-dependent phosphorylation and dephosphorylation. Although leucine degradation in skeletal muscle had been extensively studied in laboratory rats (Odessey and Goldberg, 1972; Aftring et al., 1985), it is surprising that there is very little information available on leucine degradation in skeletal muscle of avian species. A limited number of studies have suggested that the response of the degradation of BCAA and BCKA in skeletal muscle to fasting (Wijayasinghe et al., 1983) and octanoate (Wagenmakers and Veerkamp, 1984b) may be species-dependent. The objectives of this study are three-fold:

(1) to determine if fasting influences leucine degradation in chick skeletal muscle;

(2) to investigate whether changes in the leucine degradation in fasting are related to the changes in the concentrations of ketone bodies, fatty acids and glucose;

(3) to investigate the possible effect of prostaglandins on leucine degradation in chick skeletal muscle.

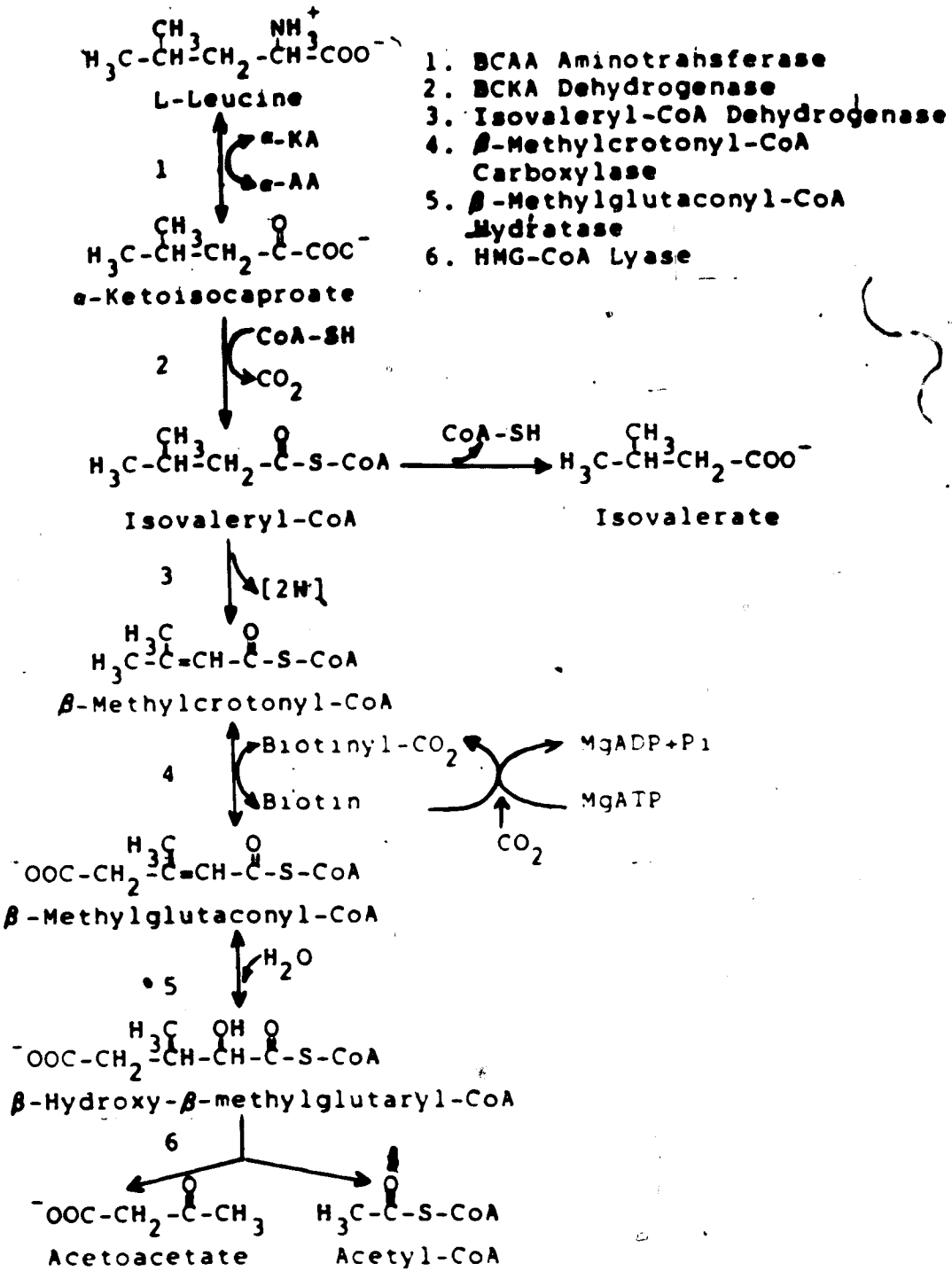

Figure I-1. The catobolic pathway of leucine

G. References

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II. The Effect of Fasting on Leucine Degradation in Chick Skeletal Muscle

A. Introduction

The branched-chain amino acids (BCAA), particularly leucine, are extensively catabolized by the skeletal musculature (Odessey and Goldberg 1972; Aftring et al. 1985). The degradation of leucine is initiated by reversible transamination to α-ketoisocaproate (KIC) by BCAA aminotransferase, followed by irreversible decarboxylation of KIC to isovaleryl-CoA by branched-chain α-ketoacid (BCKA) dehydrogenase. Skeletal muscle has relatively high BCAA aminotransferase activity and relatively low BCKA dehydrogenase activity (Shinnick and Harper 1976). Thus, it has been suggested that the activity of BCKA dehydrogenase is rate-limiting for the degradation of BCAA by skeletal muscle (Odessey and Goldberg 1979; Davis and Lee 1985). The activity of this enzyme complex can be regulated by the availability of cofactors such as NAD⁺ and CoA-SH, feedback inhibition by NADH and isovaleryl-CoA, activation by BCKA and dephosphorylation, and inactivation by ATP-dependent phosphorylation (Randle et al. 1984).

The catabolism of leucine in skeletal muscle is altered under a variety of physiological and pathological conditions (Goldberg and Odessey 1972; Goodlad and Clark 1980). For example, it is well established that leucine degradation in rat skeletal muscle is increased with fasting (Adibi at al. 1974; Wagenmakers and Veerkamp 1984). Increased leucine degradation provides energy for muscle metabolism and plays an important role in providing amino-groups for the glucose-alanine cycle (Goldberg and Chang 1978). However, Wijayasinghe et al. (1983) have shown *in vitro* with intact myofiber preparations that leucine catabolism is dramatically decreased in skeletal muscle from fasted sheep. In the 48 h fasted 20-day-old chick, Featherston and Horn (1973) reported no change in the activities of BCKA dehydrogenase in skeletal muscle homogenates. Thus, it appears that the response of leucine catabolism in skeletal muscle to food deprivation may be species-dependent. In this study, we investigated the effect of short-term fasting on leucine degradation in intact chick skeletal

muscles *in vitro*.

B. Materials and Methods

L-[U-^{14}C]-leucine and L-[1-^{14}C]-leucine were purchased from ICN Radiochemicals, Montreal, Que. Radiochemical purity was confirmed to be greater than 99% by descending paper chromatography using n-butanol:acetic acid:H_2O (2:1:1) as the solvent. Bovine insulin and all amino acids were obtained from Sigma Chemical Co., St. Louis, MO.

Day-old male broiler chicks were obtained from a local hatchery and were housed in electrically heated batteries with raised wire-mesh floors and continuous lighting. The chicks were fed ad libitum a commercial starter diet (Table II-1) until 9 days of age. On day nine, chicks weighing 115-125 g were randomly divided into three groups; group 1 was continuously fed for 24 h, group 2 was fed for 12 h and then fasted for 12 h and group 3 was fasted for 24 h. Individual chicks were anesthetised with halothane and each *extensor digitorum communis* (EDC) muscle was carefully exposed and inserted into a stainless steel wire support by means of its tendons. The EDC muscle was chosen since it has relatively long tendons at both the proximal and the distal ends and is appropriately shaped (2-2.2 cm long and no more than 0.2-0.25 cm in diameter) to optimize nutrient exchange. Each muscle was immediately dissected from the wing, weighed and placed in a test tube containing 3.5 ml Krebs-Ringer bicarbonate buffer (119 mM NaCl, 25 mM $NaHCO_3$, 4.82 mM KCl, 1.25 mM $MgSO_4$ and 1.26 mM $CaCl_2$, pH 7.4, 37°C) saturated with O_2:CO_2 (95%:5%, vol:vol). The buffer also contained 2 mM HEPES, 5 mM glucose, insulin (0.01U/ml), 0.5 mM leucine and all other amino acids at concentrations similar to those found in plasma from fed young chicks (Maruyama et al. 1976; Appendix 1). The test tube was gassed with O_2:CO_2 (95%:5%) for 1 min and sealed with a self-sealing stopper. After 30 min preincubation at 37°C in a shaking water bath (100 cycles min^{-1}), muscles from the left wings were individually transferred to 25 ml flasks containing 3 ml fresh preincubation media with L-[U-^{14}C]-leucine (300 dpm $nmol^{-1}$). Muscles from the right wings were individually transferred to 25 ml flasks containing

3 ml fresh preincubation media with L-[1-^{14}C]-leucine (300 dpm nmol^{-1}). The flasks were gassed with O_2:CO_2 (95%:5%) for 1 min and capped with self-sealing stoppers fitted with hanging plastic center-wells. The muscles were incubated in the presence of [^{14}C]-leucine for 2 h at 37°C in a shaking water bath (100 cycles min^{-1}).

The collection of $^{14}CO_2$ from oxidative decarboxylation of L-[1-^{14}C]-leucine, oxidation of L-[U-^{14}C]-leucine, and decarboxylation of [1-^{14}C]-KIC was performed as described by Aftring et al. (1985). In all instances, parallel incubations without muscles were performed to provide background values. After CO_2 collection, the center-wells were placed in 17 ml scintillation cocktail (5 g PPO, 0.2 g POPOP in 500 ml toluene plus 500 ml methyl cellosolve) and the radioactivity was measured by a scintillation spectrometer. The counting efficiency was determined to be 71% by the channels ratio method. Counts obtained in the $^{14}CO_2$ collected from L-[1-^{14}C]-leucine decarboxylation, L-[U-^{14}C]-leucine oxidation and decarboxylation of [1-^{14}C]-KIC averaged approximately 4000 cpm, 2300 cpm and 1000 cpm, respectively, for the fed birds. The corresponding background values were 45, 35 and 50 cpm, respectively. Preliminary studies showed that the recovery of $^{14}CO_2$ from [^{14}C]$NaHCO_3$ added to the incubation medium was 94.3±0.4% (mean±SEM, n=10).

Calculations of leucine catabolism were based on the specific activity of L-[1-^{14}C]-leucine and L-[U-^{14}C]-leucine in the initial incubation medium. The net rate of leucine transamination (e) was estimated by summing the rates of CO_2 production from the oxidative decarboxylation of leucine (b) and the net rate of KIC production (d). The rates of CO_2 production from the oxidation of leucine carbons 2-6 (c) was calculated as the rate of CO_2 production from the oxidation of L-[U-^{14}C]-leucine (a) minus the rate of CO_2 production from the decarboxylation of L-[1-^{14}C]-leucine (b). In addition, the percentage of transaminated leucine released as KIC and the percentage of decarboxylated leucine carbons 2-6 oxidized to CO_2 were calculated as follows:

Percentage of transaminated leucine released as KIC=(dx100%)e^{-1}

Percentage of decarboxylated leucine carbons 2-6 oxidized to $CO_2 = (cx100\%)(bx5)^{-1}$

Preliminary studies of the time-course of leucine degradation were carried out using 30 ten-day-old broiler chicks weighing 125-140 g, with six chicks for each time point. The EDC muscles were preincubated for 30 min and then incubated for periods of time ranging from 15 min to 2 h as described above. The protein content of the muscles was determined as described by Lowry et al. (1951).

The results were statistically analysed by the procedures of one-way variance analysis and the SNK multiple means comparison test using the pooled error term as described by Steel and Torrie (1980).

C. Results and Discussions

Isolated skeletal muscle preparations have been widely used for studying the regulation of leucine metabolism in the laboratory rat (e.g., Odessey and Goldberg 1972; Aftring et al. 1985) but similar preparations have not been reported to have been used for studying amino acid metabolism in avian species. The use of intact skeletal muscle preparations avoids the disadvantages associated with muscle homogenates in which the rate of oxidative decarboxylation of leucine is greatly reduced and the rate of oxidation of the remaining leucine carbon skeleton (2-6) has been reported to be essentially lost (e.g. Paul and Adibi, 1976). The rates of leucine oxidative decarboxylation ($r=0.998$, $P<0.01$), CO_2 production from total leucine oxidation ($r=0.998$, $P<0.01$) and net leucine transamination ($r=0.999$, $P<0.01$) were linear from [illegible] of incubation as shown in Fig II-1. These rates were also linear when regressed [illegible] muscle weight for muscles weighing between 16 mg and 30 mg. Regression coefficients of [illegible].92 ($P<0.01$), 0.93 ($P<0.01$) and 0.92 ($P<0.01$) were obtained for oxidative decarboxylation of leucine, CO_2 production from total leucine oxidation and net leucine transamination, respectively. These observations suggest that the activities of BCKA dehydrogenase, the tricarboxylic acid cycle and BCAA aminotransferase in the EDC muscle do not change during the 2 h incubation period and that muscle size in the

range of 16-30 mg does not differentially limit metabolic exchange under the experimental conditions used in this study. These results also indicate that the intracellular specific activity of [^{14}C]-leucine reached a plateau early during the final incubation and remained constant throughout the incubation period.

Total body weight and the weight and protein content of the EDC muscles from the fed, 12 h fasted and 24 h fasted chicks are presented in Table II-2. Total body weight of the chicks was reduced significantly ($P<0.01$) from an average of 137.6 g for the fed group to 117.1 g and 100.2 g for the 12 h fasted and 24 h fasted groups, repectively. The weight of the EDC muscles from the 24 h fasted chicks was less than that of the 12 h fasted group ($P<0.05$) and the fed group ($P<0.01$). Likewise, muscle protein content of the 24 h fasted group was also less than that of the 12 h fasted ($P<0.05$) and the fed groups ($P<0.01$). Although muscle weight of the 12 h fasted group did not differ significanly ($P>0.05$) from that of the fed group, muscle protein content of the 12 h fasted group was significantly lower ($P<0.05$) than that of the fed group. There were no significant differences ($P>0.05$) in muscle protein concentration between the fed and the fasted groups. In addition, no significant differences ($P>0.05$) in muscle weight and protein content were observed between EDC muscles from the left versus the right wings. In the laboratory rat, the decreased protein content of skeletal muscle following fasting has been attributed to both a decreased rate of protein synthesis and an increased rate of protein degradation (Fulks et al. 1975). The decrease in protein content of the chick EDC muscle following fasting resulted at least, in part, from an increased net rate of muscle protein degradation measured as an increased rate of tyrosine release (Appendix 3).

The net rate of leucine transamination in the EDC muscle from the 24 h fasted chicks was increased ($P<0.01$) by 30.4% and 38.4% as compared to EDC muscles from the 12 h fasted group and the fed group, respectively. These results are similar to those reported for quarter diaphragms obtained from 18 h fasted rats (Aftring et al. 1985), but are in contrast to results reported for intact intercostal muscle fiber bundles obtained from 5-day fasted

sheep in which net transamination of leucine was reduced by 40% (Wijayasinghe et al. 1983). The increased rate of leucine transamination is not simply a result of isotope dilution since Aftring et al. (1985) have shown that there is no significant difference in the intracellular specific activity of leucine in skeletal muscles from fed rats and fasted rats. Adibi et al. (1975) found that when fasting was prolonged to 24 h, the activity of BCAA aminotransferase in rat skeletal muscle increased two fold. They also observed an additional increase in the activity of this enzyme following a 5-day fast. Although there is little information available on the effect of fasting on BCAA aminotransferase in avian skeletal muscle, the data reported in Table II-3 indicates that the flux of leucine through the transamination step in chick skeletal muscle increases and remains greater than the flux of KIC through the oxidative decarboxylation step during fasting.

The effect of fasting on the rate of oxidative decarboxylation of leucine in the EDC muscle followed the same pattern as the effect of fasting on the net rate of transamination. As shown in Table II-3, fasting for 24 h resulted in an increase ($P<0.01$) in the rate of decarboxylation of leucine by 37.2% and 43.9% above the rate obtained with the 12 h fasted group and the fed group, respectively. These observations are consistent with those reported for skeletal muscle from 1-4 day fasted rats (Wagenmakers and Veerkamp 1984) but are in contrast to those reported for skeletal muscle fiber bundles from 5-day fasted sheep (Wijayasinghe et al. 1983) in which the rate of decarboxylation decreased by 61%. The increase in the rate of leucine oxidative decarboxylation observed in the rat and chick may be due to an increased net rate of transamination of leucine, resulting in increased formation of KIC, which in turn activates BCKA dehydrogenase by inhibiting BCKA dehydrogenase kinase (Paxton and Harris 1984). Also, increased concentrations of BCAA in skeletal muscle from both broiler chicks (Appendix 2) and rats (Adibi 1971) following short-term fasting may stimulate oxidative decarboxylation of leucine as reported in this study and in studies with rats (Paul and Adibi 1976; Aftring et al. 1985) since Aftring et al. (1986) have recently shown that leucine and isoleucine injected into rats to attain plasma concentrations within

physiological ranges activate the BCKA dehydrogenase in skeletal muscle. Studies by Odessey and Goldberg (1979) also demonstrated that increased leucine decarboxylation in rat skeletal muscle during starvation resulted from increased activity of BCKA dehydrogenase. An increase in the rate of leucine decarboxylation increases the amount of leucine carbons 2-6 available for fatty acid biosynthesis or further oxidation in the tricarboxylic acid cycle.

The rates of CO_2 production from L-[U-^{14}C]-leucine oxidation following 24 h fasting were also increased ($P<0.01$) above those obtained with the 12 h fasted group and the fed group (Table II-3). Using L-[U-^{14}C]-leucine as a tracer, Adibi et al. (1974) also reported that 24 h starvation resulted in an appreciable increase in the capacity of rat skeletal muscle to oxidise leucine as compared to the fed control and the 12 h fasted group, respectively. The increase in CO_2 production from total leucine oxidation results from both an increased rate of leucine oxidative decarboxylation and an increased rate of CO_2 production from leucine carbons 2-6 (Table II-3). These results are again in sharp contrast to the results of Wijayasinghe et al. (1983) who reported that the rates of CO_2 production from the oxidation of leucine carbons 2-6 in intercostal muscle fiber bundles from 5-day fasted sheep were reduced to only 4% of the rates obtained in the skeletal muscle fiber bundles from fed sheep. An increase in the oxidation of leucine carbons 2-6 would provide energy for muscle metabolism during fasting and would help remove isovaleryl-CoA which inhibits BCKA dehydrogenase (Randle et al. 1984).

Since the net rate of transamination of leucine exceeds the rate of leucine decarboxylation, some KIC is available to be released from the muscle to the incubation medium. Although the rate of release of KIC has been reported to increase in skeletal muscle from 5-day fasted sheep (Wijayasinghe et al. 1983) and 18-h fasted rats (Aftring et al. 1985), short-term fasting did not affect the net rate of KIC release from the chick EDC muscle (Table II-3). However, since the net rate of transamination of leucine increased, the percentage of transaminated leucine released as net KIC decreased ($P<0.01$) from an average of 28.0% and 28.7% for the fed group and the 12 h fasted group, respectively to 22.3%

following 24 h food deprivation (Table II-3). Thus, both the decrease in the percentage of transaminated leucine released as KIC and the increase in the rate of CO_2 production from leucine carbons 2-6 indicate that the oxidation of the decarboxylated leucine carbon skeleton in the tricarboxylic acid cycle is enhanced with fasting.

The percentage of decarboxylated leucine oxidized to CO_2 did not change in the EDC muscle from fasted chicks (Table II-3). In skeletal muscle fiber bundles from 5-day fasted sheep, the percentage of decarboxylated leucine oxidized to CO_2, however, has been shown to decrease from 60.8% for the fed to 8% for the fasted animals. In chick skeletal muscle, the fact that less than 50% of the decarboxylated leucine is oxidized to CO_2 suggests that oxidation of the leucine carbon skeleton in skeletal muscle is far from complete. The incomplete oxidation of leucine carbons 2-6 may be due to the low activity of isovaleryl-CoA dehydrogenase in skeletal muscle (Rhead et al. 1981) accounting for the appreciable release of isovalerate by rat skeletal muscle (Spydevold and Hokland 1983). The release of isovalerate by avian skeletal muscle has not been reported.

It is well documented that BCKA dehydrogenase is inactivated by phosphorylation by BCKA dehydrogenase kinase and activated by dephosphorylation by BCKA dehydrogenase phosphatase (Randle et al. 1984). Recent studies by Paxton and Harris (1984) have reported that acetoacetyl-CoA activates the BCKA dehydrogenase by inhibiting BCKA dehydrogenase kinase. These authors suggested that increased leucine degradation in skeletal muscle during fasting may be due in part to increased concentrations of ketone bodies, resulting in increased concentrations of acetoacetyl-CoA. Paul and Adibi (1976) have reported that physiological levels of acetoacetate stimulate CO_2 production from leucine oxidation in rat skeletal muscle homogenates. Thus, whether ketone bodies regulate leucine degradation in chick skeletal muscle merits investigation in order to understand the mechanisms of increased degradation of this essential amino acid in chick skeletal muscle during fasting.

Table II-1. Diet composition

Ingredients	Percentage (%)
Ground wheat	57.17
Stabilized animal fat	3.00
Dehydrated alfalfa meal	1.00
Meat meal (50% crude protein)	3.00
Soybean meal (40% crude protein)	28.00
Canola meal (36% crude protein)	3.00
Ground limestone	1.20
Biofos (15% Ca; 21% P)	1.00
Iodized salt	0.30
Microingredients †	2.33
Crude protein content (%)	23.00
Metabolizable energy (MJ kg^{-1})	11.98

† Supplied the following per kg of ration: manganese sulphate (27% Mn), 400 mg; zinc oxide (72% Zn), 100 mg; DL-methionine, 1.3 g; vitamin A, 4000 IU; vitamin D_3, 600 ICU; vitamin E, 10 IU; menadione, 1 mg; riboflavin, 5 mg; calcium pantothenate, 10 mg; niacin, 20 mg; choline chloride, 100 mg; folic acid, 1 mg; biotin, 100 μg; vitamin B_{12}, 10 μg; selenium, 50 μg; and amprol (25% amprolium), 500 mg.

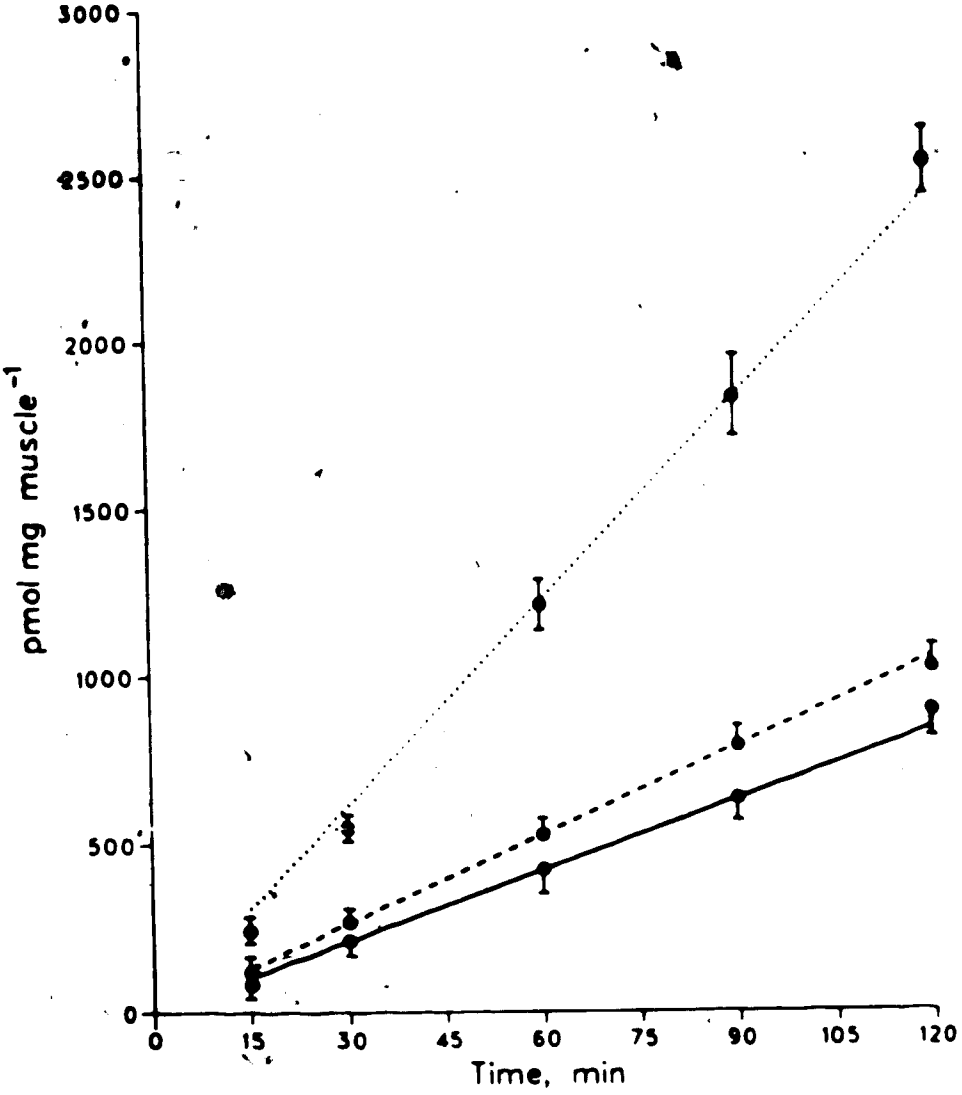

Fig. II-1. In vitro leucine degradation in EDC muscle as a function of time

..... CO_2 production from total leucine oxidation

------- net leucine transamination

_____ leucine oxidative decarboxylation

Table II-2. Body weight and extensor digitorum communis muscle weight and protein content following fasting in the ten-day-old chick.

	Fed	12 h Fast	24 h Fast
Body weight (g) †	137.6 ± 2.8 ‡A	117.1 ± 1.7 B	100.2 ± 1.1 C
EDC muscle •			
Weight (mg)	22.7 ± 1.1 Aa	20.8 ± 0.4 AB	17.0 ± 0.4 Bb
Protein (mg)	3.40 ± 0.19 Aa	2.97 ± 0.08 ABb	2.59 ± 0.05 Bc
Protein (%)	15.2 ± 0.3	14.4 ± 0.2	15.0 ± 0.1

† n=8 per treatment
‡ Mean ± SEM
• n=16 per treatment
a-c: Treatment means within rows followed by different letters a-c are significantly different ($P<0.05$).
A-C: Treatment means within rows followed by different letters A-C are significantly different ($P<0.01$).

Table II-3. In vitro degradation of leucine in the extensor digitorum communis muscle from fed and fasted chicks.

	Leucine Metabolism (nmol·h^{-1} mg muscle^{-1})						
Treatment †	CO_2 from total leucine oxidation (a)	CO_2 from leucine decarboxy-lation (b)	CO_2 from oxidation of leucine Carbons 2-6 (c=a-b)	Net KIC Production (d)	Net Trans-amination (e=b+d)	Percentage of trans-aminated leucine released as KIC (d/e x100%)e^{-1}	Percentage of leucine Carbons 2-6 oxidized to CO_2 (cx100%)(5b)$^{-1}$
Fed	1.37‡A ±0.09	0.42 A ±0.02	0.95 A ±0.07	0.16 ±0.01	0.57 A ±0.03	**28.0 A ±1.2**	**45.8 ±2.7**
Fast 12 h	1.47 A ±0.06	0.44 A ±0.02	1.04 A ±0.05	0.17 ±0.01	0.60 A ±0.02	**28.7 A ±0.9**	**48.6 ±3.4**
Fast 24 h	1.94 B ±0.10	0.60 B ±0.04	1.34 B ±0.04	0.18 ±0.01	0.79 B ±0.05	**22.3 B ±0.9**	**44.3 ±2.8**

† n=8 per treatment
‡ Mean ± SEM
A,B: Means within the column followed by different letters A,B are significantly different ($P<0.01$).

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III. Regulation of Leucine Degradation in Chick Skeletal Muscle by Ketone Bodies

A. Introduction

Ketone bodies have been shown to act as regulators of a number of biochemical events associated with the sparing of glucose and muscle proteins (Robinson and Williamson, 1980). The effects of acetoacetate and β-hydroxybutyrate on the degradation of branched-chain amino acids (BCAA), particularly leucine, in skeletal muscle have been studied with conflicting observations being reported. Buse et al.(1972) first showed that DL-β-hydroxybutyrate significantly inhibited both the decarboxylation and the total oxidation of BCAA in hemidiaphragms from fed rats. Similar effects of acetoacetate plus DL-β-hydroxybutyrate on the rates of decarboxylation of leucine have been reported in skeletal muscles from fed (Wagenmakers and Veerkamp, 1984) and fasted rats (Palmer et al., 1985). In contrast, Paul and Adibi (1978) reported that acetoacetate at concentrations ranging from 2 to 20 mM markedly increased the decarboxylation of leucine in skeletal muscle homogenates from both fed and fasted rats and suggested that increased tissue concentrations of ketone bodies may be responsible for increased leucine degradation in skeletal muscle during fasting. Thus, the role of ketone bodies in regulating leucine metabolism in skeletal muscle remains unclear.

Although ketone bodies have been implicated to regulate BCAA metabolism in rat skeletal muscle, there is no information available concerning the effects of ketone bodies on BCAA metabolism in avian skeletal muscle. In this study, we investigated the effects of acetoacetate and β-hydroxybutyrate on leucine degradation in skeletal muscle from fed and from 24-h fasted chicks, and attempted to determine whether the fasting-induced increase in leucine degradation in skeletal muscle results from the associated increase in ketone body concentrations.

B. Materials and Methods

Sodium DL-β-hydroxybutyrate (98%), lithium acetoacetate (90-95%), NADH (disodium salt), D-β-hydroxybutyrate dehydrogenase (*Rodopseudomonas spheroides*) were purchased from Sigma Chemical Co., St. Louis, MO., USA. The sources of broiler chicks, L-[1-^{14}C]leucine (52 mCi/mmol), L-[U-^{14}C]leucine (270 mCi/mmol) and other chemicals have been reported in Chapter 2.

A preliminary experiment was performed to determine the effect of fasting on plasma concentrations of β-hydroxybutyrate and acetoacetate in the chick. Thirty, 9-day-old broiler chicks weighing 115-125 g were chosen and randomly divided into three groups of 10 birds each; group 1 was continuously fed for 24 h, group 2 was fed for 12 h and then fasted for 12 h and group 3 was fasted for 24 h. Venous whole blood from the right heart of individual chicks was collected in evacuated tubes coated with heparin, mixed and immediately centrifuged at 3500 g for 15 min at 4°C. The supernatant (plasma) was stored at -70°C until analysis. Enzymatic assay of plasma D-β-hydroxybutyrate concentrations was performed using Kit# 310-A obtained from Sigma Chemical Co., St. Louis, MO., USA. Plasma acetoacetate concentrations were determined as described by Price et al. (1977).

The extensor digitorum communis (EDC) muscle was used for studies of the effects of ketone bodies on leucine metabolism in chick skeletal muscle. The EDC muscles were obtained from both fed (experiment 1) and 24-h fasted (experiment 2) 10-day-old chicks which had been allowed free access to food and water and were either fed ad libitum for 24 h or fasted for 24 h until sacrificed. The chicks were anesthetised with halothane and the EDC muscles were dissected and preincubated as described in Chapter 2. After 30 min preincubation, left and right EDC muscles were transfered to the fresh preincubation media with added L-[U-^{14}C]-leucine (300 dpm nmol^{-1}) and L-[1-^{14}C]-leucine (300 dpm nmol^{-1}), respectively, for a 2 h final incubation period as described in Chapter 2. The final incubation media contained 0, 1 or 4 mM DL-β-hydroxybutyrate or 0, 1 or 4 mM acetoacetate as indicated in Tables III-2 and III-3. The collection of $^{14}CO_2$ generated from decarboxylation

and total oxidation of leucine was performed as described in Chapter 2.

To determine whether changes in the rate of leucine degradation in the EDC muscle from fed and 24-h fasted chicks are due to changes in the uptake of leucine or in the activity of BCKA dehydrogenase, experiments 3, 4 and 5 were conducted. The Krebs-Ringer buffer incubation media in these three experements contained no glucose, amino acids or insulin since they have been shown to influence leucine degradation in rat skeletal muscle (Odessey and Goldberg, 1972; Buse et al., 1972; Hutson et al., 1980). In experiment 3, the left and right EDC muscles from fed chicks were preincubated for 30 min as described in Chapter 2 and then transferred to 2 ml Krebs-Ringer buffer (KRB) media containing L-[U-^{14}C]leucine (0.15 μCi/ml) and L-[1-^{14}C]-leucine (0.13 μCi/ml), respectively, for 1 h incubation. The KRB media contained either 0 or 4 mM DL-β-hydroxybutyrate or either 0 or 1 mM acetoacetate as indicated in Table III-4. After 1 h incubation, the muscles were thoroughly rinsed with KRB and then transferred to 3 ml KRB media alone for a 1 h final incubation. At the end of the final incubation, $^{14}CO_2$ arising from decarboxylation of L-[1-^{14}C]-leucine and from total oxidation of L-[U-^{14}C]-leucine was collected as described in Chapter 2. The radioactivity from L-[1-^{14}C]-leucine in the TCA precipitatable and TCA soluble fractions was measured as described by Odessey and Goldberg (1972). The net uptake of L-[1-^{14}C]leucine was calculated by summing the amount of radioactivity (dpm) in the final incubation medium, TCA precipitatable and TCA-soluble fractions, and $^{14}CO_2$ from the decarboxylation of L-[1-^{14}C]leucine during the final incubation.

The effects of ketone bodies on leucine decarboxylation and total oxidation were also measured in the EDC muscles from both fed (experiment 4) and 24-h fasted (experiment 5) chicks preloaded with L-[1-^{14}C]leucine or L-[U-^{14}C]leucine. The left and right EDC muscles from either fed or 24-h fasted 10-day-old broiler chicks were preincubated for 30 min as described in Chapter 2 and then transfered to 2 ml KRB media alone containing L-[U-^{14}C]-leucine (0.10 μCi/ml) and L-[1-^{14}C]-leucine (0.11 μCi/ml), respectively, for a 1 h incubation period. The media contained no insulin, glucose, amino acids or ketone bodies.

After 1 h incubation, the EDC muscles were thoroughly rinsed with KRB and then transfered to the KRB media alone containing 0 or 4 mM DL-β-hydroxybutyrate or 0, 1 mM acetoacetate for a 1 h final incubation as indicated in Tables III-5 and 6. The collection of $^{14}CO_2$ from the decarboxylation of L-[1-^{14}C]-leucine and from total oxidation of L-[U-^{14}C]-leucine was performed as described in Chapter 2.

The calculations of the rates of leucine degradation were based on the specific activity of L-[1-^{14}C] and L-[U-^{14}C]-leucine in the initial incubation media. The rates of net leucine transamination, leucine oxidative decarboxylation, CO_2 production from total leucine oxidation and the oxidation of leucine carbons 2-6 as well as the percentage of transaminated leucine released as KIC and the percentage of decarboxylated leucine carbons 2-6 oxidized to CO_2 were calculated as described in Chapter 2. Each value reported is the mean of six observations.

The results were statistically analysed by the procedures of one-way variance analysis and the SNK multiple means comparison test using the pooled error term as described by Steel and Torrie (1980).

C. Results

Plasma Concentrations of Ketone Bodies under Fed and Fasted Conditions

The plasma concentration of D-β-hydroxybutyrate in the chick increased ($P<0.01$) from 0.15 mM to 1.34 and to 2.63 mM following 12 h and 24 h fasting, respectively (Table III-1). Fasting for 12 h increased ($P<0.01$) the plasma concentration of acetoacetate by six-fold to 0.78 mM, while fasting for 24 h did not further increase ($P>0.05$) plasma concentration of acetoacetate beyond that measured after the 12 h fast.

Effect of Ketone Bodies on Leucine Degradation in Skeletal Muscle from Fed Chicks

The effect of ketone bodies on the rate of leucine degradation in skeletal muscle from fed chicks is shown in Table III-2. DL-β-hydroxybutyrate at 4 mM stimulated ($P<0.01$) the net rate of leucine transamination in EDC muscles from fed chicks by 34%, while acetoacetate at either 1 mM or 4 mM was without effect. DL-β-hydroxybutyrate at 1 mM inhibited ($P<0.05$) the rate of CO_2 production from leucine oxidative decarboxylation in the EDC muscle from the fed chicks by 29.5%. Leucine oxidative decarboxylation was not further inhibited by 4 mM DL-β-hydroxybutyrate. Acetoacetate at 1 mM and 4 mM inhibited the rate of leucine oxidative decarboxylation in the EDC muscle by 27.2% ($P<0.05$) and 47.7% ($P<0.01$), respectively. Although DL-β-hydroxybutyrate did not affect the rate of CO_2 production from total oxidation of leucine in the EDC muscle from the fed chicks, acetoacetate at 1 mM and 4 mM decreased the rate by 31.6% ($P<0.05$) and 55.6% ($P<0.01$), respectively. The rates of CO_2 production from the oxidation of leucine carbons 2-6 were also inhibited by 33.0% ($P<0.05$) and 59.6% ($P<0.01$) in the presence of 1 mM and 4 mM acetoacetate, respectively, but not in the presence of DL-β-hydroxybutyrate . Since the net rate of leucine transamination exceeds the rate of oxidative decarboxylation, net KIC is produced in skeletal muscle. DL-β-hydroxybutyrate at 4 mM markedly increased ($P<0.01$) the net rate of KIC production and the percentage of transaminated leucine released as KIC two-fold. Acetoacetate at 1 mM and 4 mM dramatically increased ($P<0.01$) the net rate of KIC production from 0.09 nmol h^{-1} mg $muscle^{-1}$ to 0.27 and 0.38 nmol h^{-1} mg $muscle^{-1}$, and increased ($P<0.01$) the percentage of transaminated leucine released as KIC from 17.1% to 44.6% and 61.5%, respectively. DL-β-hydroxybutyrate at 1 mM increased ($P<0.01$) the percentage of leucine carbons 2-6 oxidised to CO_2 in the EDC muscle from fed chicks, while acetoacetate had no significant effect.

Effect of Ketone Bodies on Leucine Degradation in Skeletal Muscle from 24-h Fasted Chicks

Both DL-β-hydroxybutyrate and acetoacetate had a more pronounced inhibiting effect on leucine catabolism in skeletal muscles from 24-h fasted chicks than in the muscles from the fed chicks as shown in Tables III-2 and III-3. DL-β-hydroxybutyrate at 1 mM and 4 mM decreased ($P<0.01$) net leucine transamination in the EDC muscle from fasted chicks by 34.2% and 54.8%, respectively. Acetoacetate at 1 mM and 4 mM inhibited ($P<0.01$) the net rate of leucine transamination in the EDC muscle from the fasted chicks by 50.7% and 57.5%, respectively. DL-β-hydroxybutyrate at 1 mM and 4 mM decreased ($P<0.01$) the rates of oxidative decarboxylation of leucine in the EDC muscle from 24-h fasted birds by 35.5% and 56.5%, respectively. The inhibiting effect of acetoacetate was more pronounced than the effect of DL-β-hydroxybutyrate, as acetoacetate at 1 mM and 4 mM inhibited ($P<0.01$) the rate of leucine decarboxylation in the muscle by 58.1% and 83.9%, respectively. The effect of both ketone bodies on the rate of CO_2 production from total oxidation of leucine followed the same pattern as their effect on the rate of leucine oxidative decarboxylation. DL-β-hydroxybutyrate at 1mM and 4 mM markedly inhibited ($P<0.01$) the rate of CO_2 production from the total oxidation of leucine by 26.8% and 51.4%, respectively, while acetoacetate at 1mM and 4 mM inhibited ($P<0.01$) this rate by 48.6% and 87.7%, respectively. The rates of CO_2 production from the oxidation of leucine carbons 2-6 were also reduced ($P<0.01$) in the presence of DL-β-hydroxybutyrate or acetoacetate at both 1 mM and 4 mM. Unlike skeletal muscle from fed chicks, neither DL-β-hydroxybutyrate nor acetoacetate at 1 mM influenced the rates of net KIC production in skeletal muscles from 24-h fasted chicks whereas acetoacetate at 4 mM increased ($P<0.01$) this rate by 120%. Although DL-β-hydroxybutyrate at 1 mM and 4 mM did not affect the percentage of transaminated leucine released as KIC in the muscles from the fasted birds, the value was increased from 13.9% to 26.1% and 69.4% in the presence of 1 mM and 4 mM acetoacetate, respectively. In addition, 4 mM acetoacetate inhibited ($P<0.01$) the percentage of leucine carbons 2-6 oxidized to CO_2 by 34.1%, while DL-β-hydroxybutyrate had no effect.

Effect of Ketone Bodies on Net Leucine Uptake by Skeletal Muscle from Fed Chicks

When EDC muscles from fed chicks were incubated with L-[1-^{14}C]leucine in the presence of either 4 mM DL-β-hydroxybutyrate or 1 mM acetoacetate the net uptake of L-[1-^{14}C]leucine increased ($P<0.01$) above that in control muscles (Table III-4). However, the production of $^{14}CO_2$ from the oxidative decarboxylation of L-[1-^{14}C]leucine was decreased ($P<0.05$) in the EDC muscle during the subsequent 1 h incubation in the absence of ketone bodies and labelled leucine (Table III-4). The production of $^{14}CO_2$ from the total oxidation of L-[U-^{14}C]leucine in the final incubation period did not change significantly in EDC muscles previously incubated in the presence of 4 mM DL-β-hydroxybutyrate, but decreased ($P<0.01$) in EDC muscles previously incubated in the presence of 1 mM acetoacetate before transfer to the final incubation media. Table III-4 also shows that the incorporation of L-[1-^{14}C]leucine into skeletal muscle TCA precipitatable protein was increased ($P<0.05$) in the presence of either 4 mM DL-β-hydroxybutyrate or 1 mM acetoacetate.

Effect of Ketone Bodies on Leucine Degradation in Fed and Fasted Chick Skeletal Muscle Preloaded with [^{14}C]-Leucine

When the EDC muscles from fed chicks were preloaded with L-[1-^{14}C]-leucine or L-[U-^{14}C]-leucine by incubating in the presence of L-[1-^{14}C]-leucine or L-[U-^{14}C]-leucine in the absence of ketone bodies for 1 h and then transferred to the KRB media containing either 4 mM DL-β-hydroxybutyrate or 1 mM acetoacetate, the production of $^{14}CO_2$ from the oxidative decarboxylation of L-[1-^{14}C]leucine was decreased ($P<0.01$) by 24.1% and 32.6% below control values, respectively (Table III-5). The incorporation of L-[1-^{14}C]leucine into TCA precipitatable muscle protein was not influenced by the presence of ketone bodies in the final incubation media. The production of $^{14}CO_2$ from the total oxidation of L-[U-^{14}C]-leucine did not significantly change in the presence of 4 mM DL-β-hydroxybutyrate and 1 mM acetoacetate (Table III-5). Similar effects of both 4 mM DL-β-hydroxybutyrate and 1 mM acetoacetate on the production of $^{14}CO_2$ from

decarboxylation of L-[1-^{14}C]leucine, total oxidation of L-[U-^{14}C]leucine and on the amount of L-[1-^{14}C]-leucine incorporated into TCA precipitatable protein in EDC muscles from 24-h fasted chicks were observed (Table III-6).

D. Discussion

Little information is available concerning the regulation of leucine transamination in intact skeletal muscle by ketone bodies. Although the activity of BCAA aminotransferase is relatively high in skeletal muscle, leucine transamination can be affected by the availability of amino group acceptors such as α-ketoglutarate and pyruvate (Aftring et al., 1985). In this study, we found that net leucine transamination in chick skeletal muscle is also subject to regulation by ketone bodies depending on the physiological state of the animal. DL-β-hydroxybutyrate at 4 mM stimulated the net rate of leucine transamination in skeletal muscle from fed chicks, while acetoacetate had no effect (Table III-2). Possibly, increasing intracellular concentrations of NADH in muscles from fed chicks increases the net rate of leucine transamination since the metabolism of β-hydroxybutyrate increases NADH to a greater extent than does acetoacetate (Robinson and Williamson, 1980). However, both DL-β-hydroxybutyrate (4 mM) and acetoacetate (1 mM) markedly inhibited the net rate of leucine transamination in the EDC muscle from 24-h fasted chicks (Table III-3). The inhibition of the net rate of leucine transamination by ketone bodies may result from either decreased activity of BCAA aminotransferase or decreased availability of amino group acceptors such as pyruvate and α-ketoglutarate or both. The results obtained from this study may partly explain why the release of alanine and glutamine by skeletal muscles from fed rats was not changed in the presence of acetoacetate (Berger et al., 1980), and the release of these two amino acids by skeletal muscle from fasted rats was decreased in the presence of ketone bodies (Palaiologos and Felip, 1976) on the basis that amino groups for the *de novo* synthesis of alanine and glutamine mainly come from the transamination of BCAA in skeletal muscle (Goldberg and Chang, 1978).

Reports in the literature on the effect of ketone bodies on the oxidative decarboxylation of leucine in skeletal muscles from fed and fasted rats are variable. DL-β-hydroxybutyrate (1 mM) has been shown to have no effect on leucine decarboxylation in hemidiaphragms from fed rats (Odessey and Goldberg, 1972) whereas $^{14}CO_2$ production from L-[1-^{14}C]leucine has been reported to be inhibited by 4 mM DL-β-hydroxybutyrate in hemidiaphragms from fed rats (Buse et al., 1967), and by 4 mM DL-β-hydroxybutyrate plus 1 mM acetoacetate in hemidiaphragms from fed (Wagenmakers and Veerkamp, 1984) and 40-h fasted rats (Palmer et al., 1985). In contrast, Paul and Adibi (1978) found that 2-20 mM acetoacetate markedly stimulated leucine oxidative decarboxylation in homogenates of skeletal muscle from both fed and fasted rats. Based on their observations, Paul and Adibi (1978) suggested that an increase in ketone body concentration may result in an increase in the rate of leucine degradation in skeletal muscle during fasting. This hypothesis is consistant with the recent findings that acetoacetyl-CoA, which arises from the metabolism of ketone bodies in skeletal muscle (Robinson and Williamson, 1980), activates BCKA dehydrogenase by inhibiting BCKA dehydrogenase kinase (Paxton and Harris, 1984). In the present studies, however, we found that physiological concentrations of β-hydroxybutyrate and acetoacetate (Tabel III-1) caused a marked inhibition of leucine oxidative decarboxylation in skeletal muscle from both fed and fasted chicks regardless of the presence or absence of insulin, amino acids or glucose (Tables III 2-6). These results are in contrast to the recent observation by Palmer et al. (1985) [redacted] inhibition by ketone bodies of leucine oxidative decarboxylation in incubated hemidiaphragms from 40-h fasted rats is glucose-dependent. Unlike glucose which inhibits the rate of leucine decarboxylation in the EDC muscle from fed chicks but has no effect in the EDC muscle from 24-h fasted chicks (Chapter 4), the inhibition of leucine decarboxylation by ketone bodies is even more pronounced in skeletal muscle from 24-h fasted chicks (Table III-3). Recent studies *in vivo* have demonstrated that there is an inverse relationship between oxidative decarboxylation of leucine and KIC and plasma levels of ketone bodies (Tessari et al., 1986; Beaufrere et al., 1985). In addition, it has

been shown by Reeds et al. (1981) that the oxidative decarboxylation of leucine *in vivo* is decreased when young pigs are fed a high fat diet which results in high concentrations of ketone bodies. It is therefore probable that the increased leucine oxidative decarboxylation in skeletal muscle from fasted animals is not likely due to increased plasma concentrations of β-hydroxybutyrate and acetoacetate.

The inhibiting effect of ketone bodies on leucine oxidative decarboxylation is independent of leucine uptake since the $^{14}CO_2$ production from L-[1-^{14}C]-leucine in EDC muscles from both fed and 24-h fasted chicks is inhibited, although the net uptake of ^{14}C-leucine is the same (Tables III-5 and 6). The decreased production of $^{14}CO_2$ from decarboxylation of L-[1-^{14}C]-leucine in the presence of ketone bodies is not likely due to dilution of intracellular L-[1-^{14}C]-leucine based on the following evidence: (1) both DL-β-hydroxybutyrate and acetoacetate did not affect the incorporation of L-[1-^{14}C]-leucine into TCA precipitatable protein in EDC muscles from both fed and 24-h fasted chicks (Tables III-5 and 6); (2) both DL-β-hydroxybutyrate and acetoacetate had no effect on the net rate of protein degradation in EDC muscles from fed chicks as measured by tyrosine release (Appendix 3); (3) both DL-β-hydroxybutyrate and acetoacetate inhibited the net rate of protein degradation in the EDC muscles from 34-h fasted chicks (Appendix 2), therefore the intracellular L-[1-^{14}C]-leucine would likely be less diluted in muscles from fasted chicks as compared to the muscles from fed chicks. In the latter case, the inhibition of $^{14}CO_2$ production from the decarboxylation of L-[1-^{14}C]-leucine by ketone bodies may be underestimated. β-hydroxybutyrate inhibits leucine oxidative decarboxylation probably by increasing intracellular concentration of NADH and decreasing the availability of NAD$^+$ and CoA-SH since NADH and both CoA-SH and NAD$^+$ have been shown to be an inhibitor and cofactors of BCKA dehydrogenase, respectively (Randle et al., 1984). Similarly, acetoacetate inhibits the decarboxylation of leucine probably by decreasing the availability of NAD$^+$ and CoA-SH.

The net rate of KIC production depends upon both the net rate of leucine transamination and the rate of KIC oxidative decarboxylation. The effect of ketone bodies on KIC production in skeletal muscle has not been previously reported. In this study, it was found that 4 mM DL-β-hydroxybutyrate increased the net rate of KIC production four-fold in skeletal muscle from fed chicks (Table III-2). This increase was apparently due to an increased net rate of leucine transamination and a decreased rate of leucine oxidative decarboxylation in the presence of 4 mM DL-β-hydroxybutyrate. Although 1 mM and 4 mM acetoacetate had no significant effect on the net rate of leucine transamination in muscles from fed chicks, the decreased rate of leucine oxidative decarboxylation was likely responsible for the three to four fold increase in the net rate of KIC production (Table III-2). Accordingly, the percentage of transaminated leucine released as KIC was increased in muscles incubated in the presence of 4 mM DL-β-hydroxybutyrate as well as 1 mM and 4 mM acetoacetate. On the other hand, it is interesting to note that in skeletal muscle from 24-h fasted chicks, 4 mM DL-β-hydroxybutyrate and 1 mM acetoacetate had no effect on the net rate of KIC production, but 4 mM acetoacetate still increased the rate two-fold due to further inhibition of leucine oxidative decarboxylation (Table III-3). The percentage of transaminated leucine released as KIC was thus increased in the presence of 1 mM and 4 mM acetoacetate since the net rate of KIC production did not change (1 mM acetoacetate) or increased (4 mM acetoacetate) in spite of a much lower net rate of leucine transamination (Table III-3). In fasted chick muscles incubated in the presence of 4 mM DL-β-hydroxybutyrate and 1 mM acetoacetate, the net rate of leucine transamination was only slightly higher than the rate of leucine oxidative decarboxylation, thus accounting for the small amount of KIC available for release into the incubation media.

The rate of CO_2 production from total oxidation of leucine is the sum of the rate of leucine oxidative decarboxylation and the rate of CO_2 production from the oxidation of leucine carbons 2-6 in the tricarboxylic acid cycle. Although DL-β-hydroxybutyrate inhibited the rate of leucine oxidative decarboxylation in skeletal muscle from fed chicks, the rate of

CO_2 production from the total oxidation of leucine did not significantly change. This may be due to either less dilution of [U-^{14}C]-acetyl-CoA by unlabelled acetyl-CoA from oxidation of glucose and fatty acids or inhibition of the steps beyond the α-decarboxylation such as dehydrogenation of isovaleryl-CoA by isovaleryl-CoA dehydrogenase which has low activity in skeletal muscle (Rhead et al., 1982). In EDC muscles from fed chicks incubated with acetoacetate and from 24-h fasted chicks incubated with either DL-β-hydroxybutyrate or acetoacetate, decreased rates of CO_2 production from total leucine oxidation (Tables III-2 and 3) result from both decreased rates of leucine decarboxylation and decreased rates of CO_2 production from oxidation of leucine carbons 2-6. Acetoacetate has a more pronounced inhibiting effect on the rate of CO_2 production from the oxidation of leucine carbons 2-6 in EDC muscles from both fed and 24-h fasted chicks (Tables III-2 and 3) than does DL-β-hydroxybutyrate. These results may be due to the fact that acetoacetate undergoes more extensive oxidation in skeletal muscle from both fed and fasted animals than does β-hydroxybutyrate (Ruderman et al., 1971; Ruderman and Goodman, 1973; Maizels et al., 1977).

It is interesting to note that the effect of ketone bodies on $^{14}CO_2$ production from total oxidation of L-[U-^{14}C]leucine depends on the experimental conditions. Acetoacetate inhibited the production of $^{14}CO_2$ from oxidation of L-[U-^{14}C]leucine in skeletal muscle from both fed and fasted chicks in the presence of insulin, glucose and amino acids (Tables III-2,3) but had no effect in the absence of insulin, glucose and amino acids (Tables III 5,6). However, when the muscle from the fed chick was incubated with acetoacetate for 1 h in the KRB medium containing L-[U-^{14}C]leucine but no insulin, glucose or amino acids and then transferred to the same KRB media for another hour of incubation, appreciable inhibition of $^{14}CO_2$ production from L-[U-^{14}C]leucine was observed (Table III-4). These results suggest that the effect of ketone bodies on $^{14}CO_2$ production from the total oxidation of L-[U-^{14}C]leucine is independent of insulin, glucose or amino acids although the metabolic reasons for these observations are not understood.

The present studies suggest that under certain conditions, a decreased flux of leucine through BCAA aminotransferase or a decreased flux of KIC through BCKA dehydrogenase can limit the degradation of leucine in skeletal muscle (Tables III 2,3). In addition, the rate of leucine transamination does not seem to control the total oxidation of leucine since the presence of 4 mM DL-β-hydroxybutyrate inhibited the rate of leucine decarboxylation and the rates of CO_2 production from the oxidation of leucine carbons 2-6 despite the increase in the net rate of leucine transamination in skeletal muscle from fed chicks (Table III-2). These results indicated that BCKA dehydrogenase is rate-limiting for the degradation of leucine in chick skeletal muscle as in rat skeletal muscle as suggested by Odessey and Goldberg (1979). It is interesting that although plasma concentrations of both β-hydroxybutyrate and acetoacetate (Table III-1) are increased during fasting, the apparent flux of leucine through the BCAA aminotransferase and the flux of KIC through the BC[illegible]ogenase in skeletal muscle is not inhibited but accelerated at least in 24 h fasti[illegible]2). It remains to be determined how leucine degradation in skeletal muscle is e[illegible]ring fasting.

Table III-1. The effect of fasting on the concentration of ketone bodies in plasma from the ten-day-old chick

Treatment †	DL-β-hydroxybutyrate (mM)	Acetoacetate (mM)
Fed	0.15 ± 0.002 ‡A	0.11 ± 0.01 A
12-h Fast	1.34 ± 0.009 B	0.78 ± 0.04 B
24-h Fast	2.63 ± 0.016 C	0.89 ± 0.05 B

† n=10 per treatment
‡ Mean ± SEM
A-C: Treatment means within the column followed by different letters A-C are significantly different ($P<0.01$).

Table III-2. The effect of DL-β-hydroxybutyrate and acetoacetate on leucine degradation in skeletal muscle from fed chicks.

	Leucine Metabolism (nmol h^{-1} mg muscle $^{-1}$)						
Treatment†•	CO_2 from total leucine oxidation	CO_2 from leucine decarboxy-lation	CO_2 from oxidation of leucine Carbons 2-6	Net KIC Production	Net Trans-amination	Percentage of trans-aminated leucine released as KIC	Percentage of leucine Carbons 2-6 oxidized to CO_2
	(a)	(b)	(c=a-b)	(d)	(e=b+d)	(dx100%)e^{-1}	(cx100%)$(5b)^{-1}$
Control	1.33‡Aa ±0.14	0.44 Aa ±0.05	0.88 Aa ±0.08	0.09 Ac ±0.01	0.53 Aab ±0.05	**17.1 Aa ±1.8**	**40.1 BCab ±2.9**
1 mM HB	1.16 Aab ±0.08	0.31 ABb ±0.03	0.85 Aa ±0.05	0.10 Ac ±0.01	0.42 Aa ±0.04	**24.4 Aa ±2.8**	**57.4 Ac ±5.1**
4 mM HB	1.16 Aab ±0.06	0.33 ABb ±0.02	0.83 Aa ±0.05	0.38 Ba ±0.04	0.71 Bc ±0.03	**52.5 BCbc ±3.9**	**51.4 ABbc ±4.5**
1 mM AcAc	0.91 ABb ±0.12	0.32 ABb ±0.02	0.59 ABb ±0.11	0.27 Bb ±0.03	0.59 ABbc ±0.03	**44.6 Bb ±3.8**	**35.5 BCa ±5.1**
4 mM AcAc	0.59 Bc ±0.05	0.23 Bb ±0.01	0.36 Bc ±0.06	0.38 Ba ±0.04	0.61 ABbc ±0.04	**61.5 Cc ±2.0**	**31.1 Ca ±5.1**

† n=6 per treatment
‡ Mean ± SEM
• Incubation media contained insulin, amino acids and glucose.
HB: DL-β-hydroxybutyrate AcAc: acetoacetate
a, b, c: Means followed by different letters a, b, c within the column are significantly different ($P<0.05$).
A, B, C: Means followed by different letters A, B, C within the column are significantly different ($P<0.01$)

Table III-3. The effect of DL-β-hydroxybutyrate and acetoacetate on leucine degradation in skeletal muscle from 24-h fasted chicks.

	Leucine Metabolism (nmol h^{-1} mg muscle^{-1})						
Treatment†*	CO_2 from total leucine oxidation (a)	CO_2 from leucine decarboxylation (b)	CO_2 from oxidation of leucine carbons 2-6 (c=a-b)	Net KIC Production (d)	Net Transamination (e=b+d)	Percentage of transaminated leucine released as KIC (dx100%)c^{-1}	Percentage of leucine carbons 2-6 oxidized to CO_2 (cx100%)$(5b)^{-1}$
Control	1.79‡A ±0.06	0.62 A ±0.04	1.17 A ±0.07	0.10 A ±0.01	0.72 Aa ±0.04	**13.9 Aa ±1.2**	**38.4 A ±4.2**
1 mM HB	1.31 B ±0.05	0.40 B ±0.02	0.91 B ±0.03	0.08 A ±0.01	0.48 Bb ±0.03	**15.6 Aa ±2.0**	**45.6 A ±3.1**
4 mM HB	0.87 C ±0.06	0.27 C ±0.03	0.60 C ±0.04	0.06 A ±0.01	0.33 Bc ±0.04	**17.8 Aa ±2.4**	**45.7 A ±2.9**
1 mM AcAc	0.92 C ±0.08	0.26 C ±0.02	0.66 C ±0.07	0.09 A ±0.01	0.36 Bc ±0.02	**26.1 Ab ±3.2**	**49.7 A ±4.4**
4 mM AcAc	0.22 D ±0.03	0.10 D ±0.01	0.12 D ±0.02	0.22 B ±0.02	0.31 Bc ±0.03	**69.4 Bc ±3.5**	**25.3 B ±3.1**

† n=6 per treatment
‡ Mean ± SEM
* Incubation media contained insulin, amino acids and glucose.
HB: DL-β-hydroxybutyrate AcAc: acetoacetate
a, b, c: Means followed by different letters a, b, c within the column are significantly different ($P<0.05$).
A, B, C: Means followed by different letters A, B, C within the column are significantly different ($P<0.01$)

Table III-4. The effect of ketone bodies on leucine uptake and metabolism in EDC muscle from fed chicks

Incubation with [^{14}C]-leucine †*	Uptake of L-[1-^{14}C]-leucine	L-[1-^{14}C]-leucine in TCA-insoluble fraction	$^{14}CO_2$ from L-[1-^{14}C]-leucine	$^{14}CO_2$ from L-[U-^{14}C] leucine
Buffer (KRB)	10062 ‡ A ±284	7256 a ±356	1225 a ±74	1606 Aa ±116
Buffer + 4 mM HB	11979 B ±785	8750 b ±262	990 b ±63	1364 ABa ±98
Buffer + 1 mM AcAc	11875 B ±501	8781 b ±499	985 b ±65	1067 Bb ±92

† n=10 per treatment
‡ Values are expressed as dpm (23 mg muscle)$^{-1}$ and given as mean ± SEM.
* Incubation media contained no insulin, amino acids or glucose.
HB: DL-β-hydroxybutyrate; AcAc: acetoacetate.
a,b: Means within the column followed by different letters a,b are significantly different ($P<0.05$).
A,B: Means within the column followed by different letters A,B are significantly different ($P<0.01$).

Table III-5. The effect of ketone bodies on leucine metabolism in fed chick EDC muscles preloaded with [^{14}C]-leucine in vitro

Final 1 h incubation †•	Uptake of L-[1-^{14}C]-leucine	L-[1-^{14}C]-leucine in TCA-insoluble fraction	$^{14}CO_2$ from L-[1-^{14}C]-leucine	$^{14}CO_2$ from L-[U-^{14}C] leucine
Buffer (KRB)	8895 ‡ ±422	5681 ±357	1104 A ±35	1234 ±44
Buffer + 4 mM HB	9164 ±376	6525 ±413	838 B ±26	1071 ±52
Buffer + 1 mM AcAc	9071 ±458	6328 ±473	744 B ±59	1150 ±79

† n=10 per treatment
‡ Values are expressed as dpm (23 mg muscle)$^{-1}$ and given as mean ± SEM.
• Incubation media contained no insulin, amino acids or glucose.
HB: DL-β-hydroxybutyrate; AcAc: acetoacetate.
A,B: Means within the column followed by different letters A,B are significantly different ($P<0.01$).

Table III-6. The effect of ketone bodies on leucine metabolism in 24-h fasted chick EDC muscles preloaded with [^{14}C]-leucine in vitro

Final 1 h incubation †•	Uptake of L-[1-^{14}C]-leucine	L-[1-^{14}C]-leucine in TCA-insoluble fraction	$^{14}CO_2$ from L-[1-^{14}C]-leucine	$^{14}CO_2$ from L-[U-^{14}C] leucine
Buffer (KRB)	5560 ‡ ±501	3636 ±322	704 A ±42	1944 ±115
Buffer + 4 mM HB	5304 ±439	3507 ±229	497 B ±32	1672 ±42
Buffer + 1 mM AcAc	5356 ±458	3528 ±473	528 B ±59	1729 ±79

† n=10 per treatment
‡ Values are expressed as dpm (17 mg muscle)$^{-1}$ and given as mean ± SEM.
• Incubation media contained no insulin, amino acids or glucose.
HB: DL-β-hydroxybutyrate; AcAc: acetoacetate.
A,B: Means within the column followed by different letters A,B are significantly different ($P<0.01$).

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IV. Effect of Octanoate and Glucose on Leucine Degradation in Skeletal Muscle from Fed and Fasted Chicks

A. Introduction

The initial step in the degradation of leucine is reversible transamination with α-ketoglutarate to α-ketoisocaproate (KIC) catalysed by branched-chain amino acid (BCAA) aminotransferase, followed by irreversible decarboxylation of KIC to isovaleryl-CoA catalysed by branched-chain α-ketoacid (BCKA) dehydrogenase. Since the activity of BCAA aminotransferase is relatively high and the activity of BCKA dehydrogenase is relatively low in skeletal muscle (Shinnick and Harper, 1976), it has been suggested that BCKA dehydrogenase is rate-limiting for the degradation of BCAA in skeletal muscle (Odessey and Goldberg, 1979). The degradation of leucine in skeletal muscle from fed rats in which the activation of BCKA dehydrogenase is low (W[illegible]ers and Veerkamp, 1984) has been shown to be stimulated by insulin (Manchester, 196[illegible]), long-chain and medium-chain fatty acids such as palmitate and octanoate (Buse et al., 1972) but inhibited by ketone bodies (Wagenmakers and Veerkamp, 1984), glucose (Odessey and Goldberg, 1972; Buse et al., 1972) and pyruvate (Aftring et al., 1985).

Recent studies with the perfused rat heart have shown that octanoate may either activate or inhibit the rate of oxidative decarboxylation of leucine and KIC depending on the initial activity of BCKA dehydrogenase. These observations imply that insulin, fatty acids, ketone bodies and glucose may have different effects on leucine degradation in skeletal muscle from fasted rats in which BCKA dehydrogenase is relatively activated (Odessey and Goldberg, 1979) as opposed to muscles from fed rats in which only a small proportion of BCKA dehydrogenase is in the active form (Wagenmakers and Veerkamp, 1984). This hypothesis, however, has not been tested in skeletal muscle. We chose octanoate and glucose as examples of activators and inhibitors, respectively, of BCKA decarboxylation in skeletal muscle from fed rats to determine their effects on metabolism in skeletal muscle from fed and fasted chicks. The young broiler chick was used as the animal model as there is very little

information available concerning leucine metabolism in avian species.

B. Materials and Methods

Octanoate (99%), bovine insulin, glucose and amino acids were obtained from Sigma Chemical Co., St. Louis, MO., USA. The sources of broiler chicks , L-[1-^{14}C]-leucine and L-[U-^{14}C]-leucine have been reported in Chapter 2.

For studies of the effects of octanoate and glucose on leucine degradation in skeletal muscle from fed and 24-h fasted chicks, nine-day-old broiler chicks weighing between 125-140 g were either provided feed *ad libitum* for 24 h or fasted for 24-h. The chicks were anesthetized and the left and right extensor digitorum communis (EDC) muscles were dissected and preincubated for 30 min as described in Chapter 2. The left and right EDC muscles were then transferred to fresh incubation media with added insulin, amino acids and L-[U-^{14}C]-leucine (300 dpm nmol^{-1} and L-[1-^{14}C]-leucine (300 dpm nmol^{-1}), respectively, and incubated for 2 h as described in Chapter 2. The final incubation media contained octanoate in 0, 0.2 or 1 mM concentration in the presence of 5 mM glucose or glucose in 0, 5 or 12 mM concentration. Octanoate was neutralized with NaOH before addition to the incubation medium. The collection of $^{14}CO_2$ liberated from the decarboxylation of L-[1-^{14}C]leucine, total oxidation of L-[U-^{14}C]leucine and the decarboxylation of [^{14}C]KIC was performed as described in Chapter 2. The rate of leucine transamination, leucine oxidative decarboxylation, CO_2 production from total leucine oxidation and the oxidation of leucine carbons 2-6 as well as the percentage of transaminated leucine released as KIC and the average percentage of leucine carbons 2-6 oxidized to CO_2 were calculated as described in Chapter 2.

The results were statistically analysed by the procedures of one-way variance analysis and SNK multiple means comparison test using the pooled error term as described by Steel and Torrie (1980).

C. Results

Effect of Octanoate on Leucine Degradation in Skeletal Muscle from Fed and Fasted Chicks

The effects of octanoate on leucine degradation in skeletal muscle from fed chicks is presented in Table IV-1. The net rates of leucine transamination in the EDC muscle from fed chicks increased ($P<0.01$) from 0.54 nmol h^{-1} mg $muscle^{-1}$ in the absence of octanoate to 0.71 and 0.83 nmol h^{-1} mg $muscle^{-1}$ in the presence of 0.2 mM and 1 mM octanoate respectively. This fatty acid at 0.2 mM and 1 mM increased ($P<0.01$) the rates of leucine oxidative decarboxylation in EDC muscle from fed chicks from 0.41 nmol h^{-1} mg $muscle^{-1}$ for the control group to 0.56 and 0.71 nmol h^{-1} mg $muscle^{-1}$, respectively. Octanoate at 0.2 mM increased ($P<0.01$) the rate of CO_2 production from [illegible] leucine oxidation from 1.40 nmol h^{-1} mg $muscle^{-1}$ for the control group to 1.81 nmol [illegible] mg $muscle^{-1}$, and at 1 mM further increased ($P<0.01$) this rate to 2.36 nmol h^{-1} [illegible] $muscle^{-1}$. The rates of CO_2 production from oxidation of leucine carbons 2-6 were also increased ($P<0.01$) from 0.97 nmol h^{-1} mg $muscle^{-1}$ for the control group to 1.24 and 1.65 nmol h^{-1} mg $muscle^{-1}$ in the presence of 0.2 and 1 mM octanoate, respectively. Octanoate at either 0.2 mM or 1 mM did not influence ($P>0.05$) the net rates of KIC production by the EDC muscles from fed chicks. Due to increased net rates of leucine transamination and no change in the net rates of KIC production, octanoate at 1 mM decreased ($P<0.01$) the percentage of transaminated leucine released as KIC from 23.9% for the control group to 14.2%, but this fatty acid at 0.2 mM had no significant effect on this parameter. Octanoate at both 0.2 mM and 1 mM did not influence ($P>0.05$) the percentage of leucine carbons 2-6 oxidized to CO_2.

A comparison of the values presented in Tables IV-1 and IV-2 indicates that fasting the chicks for 24 h increases the rates of leucine degradation in the EDC muscle. The fed and fasted control values are similar to those reported in Chapter 2. The inclusion of octanoate in the incubation media at either 0.2 mM or 1 mM had no effect on any of the parameters of leucine metabolism measured in muscles from the 24-h fasted birds (Table IV-2).

Effect of Glucose on Leucine Degradation in Skeletal Muscle from Fed and Fasted Chicks

The effect of glucose on the rate of leucine degradation in skeletal muscle from the fed chicks is shown in Table IV-3. The net rates of leucine transamination were not significantly altered ($P>0.05$) by increasing the concentration of glucose in the incubation medium from 0 to 5 or 12 mM. However, the rate of leucine oxidative decarboxylation was decreased ($P<0.01$) from 0.56 nmol h^{-1} mg $muscle^{-1}$ in the absence of glucose to 0.41 and 0.39 nmol h^{-1} mg $muscle^{-1}$ in the presence of 5 and 12 mM glucose, respectively. Also, the rate of CO_2 production from total leucine oxidation was decreased ($P<0.01$) from 2.06 nmol h^{-1} mg $muscle^{-1}$ for the control to 1.44 and 1.35 nmol h^{-1} mg $muscle^{-1}$ in the presence of 5 and 12 mM glucose, respectively. Similarly, the addition of 5 and 12 mM glucose into the incubation medium decreased ($P<0.01$) the rate of CO_2 production from the oxidation of decarboxylated leucine carbons 2-6 from 1.50 nmol h^{-1} mg $muscle^{-1}$ for the control to 1.03 and 0.96 nmol h^{-1} mg $muscle^{-1}$, respectively. Since the net rates of leucine transamination did not change and the rates of leucine oxidative decarboxylation decreased, the addition of 5 and 12 mM glucose increased the rates of net KIC production from 0.09 nmol h^{-1} mg $muscle^{-1}$ for the control to 0.12 ($P<0.05$) and 0.18 nmol h^{-1} mg $muscle^{-1}$ ($P<0.01$). The percentage of transaminated leucine released as KIC was increased ($P<0.01$) from 10.4% for the control to 22.2% and 31.3% in the presence of 5 and 12 mM glucose, respectively. The percentage of decarboxylated leucine carbons oxidized to CO_2 was not altered in the presence of glucose in the EDC muscle from fed chicks.

The effect of the addition of glucose to the incubation media on the rate of leucine degradation in skeletal muscle from 24-h fasted chicks is presented in Table IV-4. In contrast to muscles from fed chicks, the rates of leucine oxidative decarboxylation, CO_2 production from total leucine oxidation and the oxidation of decarboxylated leucine carbons 2-6 oxidized to CO_2 were not decreased in response to addition of glucose to the incubation media. Also, the addition of glucose to the incubation medium did not influence ($P>0.05$) the net rate of leucine transamination, the net rate of KIC production or the percentage of transaminated

cine released as KIC.

D. Discussion

Few studies to determine the effect of octanoate on the net rate of leucine transamination in skeletal muscle have been reported. In the quarter-diaphragm from fed rats incubated with 1 mM octanoate, Wagenmakers and Veerkamp (1984) reported no change in the net rate of leucine transamination. However, the present studies show a dramatic increase in the net rate of leucine transamination in the intact EDC muscle from fed chicks incubated in the presence of 0.2 and 1 mM octanoate. Since octanoate has been shown not to affect leucine uptake by skeletal muscles (Buse et al., 1972), it is possible that the increased net rate of leucine transamination may be due to increased intracellular concentrations of leucine because octanoate has been shown to inhibit the incorporation of leucine into muscle protein (Wagenmakers and Veerkamp, 1984) and to have no effect on muscle protein degradation (Fulks et al., 1975). In contrast to EDC muscles from fed chicks, octanoate did not affect the net rate of leucine transamination in muscles from 24-h fasted chicks (Table IV-2). In fact, the net rate of leucine transamination in muscle from fasted chicks was equal to that in muscle from fed chicks incubated in the presence of 1 mM octanoate. This implies that under conditions such as fasting in which the rate of net leucine transamination is elevated, further stimulation by potent activators such as octanoate is not achieved. These observations suggest that the stimulating effects of octanoate and fasting on the net rate of leucine transamination in skeletal muscle are not additive.

It is well documented that octanoate promotes the rate of leucine oxidative decarboxylation in skeletal muscle from fed rats. In this study, a similar response is also reported for skeletal muscles from fed chicks (Table IV-1). The mechanisms involved, however, are not understood. Based on the observations that the release of isovalerate by skeletal muscle was increased (Spydevold and Hokland, 1983) and that $^{14}CO_2$ production from [U-^{14}C]KIC did not change in the presence of 1 mM octanoate (Wagenmakers and Veerkamp,

1984). Wagenmakers and Veerkamp (1984) suggested that the exchange of octanoyl-carnitine and branched-chain acylcarnitine via the carnitine-acylcarnitine translocase may help stimulate the oxidative decarboxylation of KIC by increasing the efflux of mitochondrial isovaleryl-CoA into the cytoplasm, thus reducing the amount available to inhibit BCKA dehydrogenase (Randle et al., 1984). The results from the present study imply that the transport of isovaleryl-CoA from the mitochondrion to the cytoplasm is not changed in skeletal muscle from fed chicks in the presence of octanoate since the rates of $^{14}CO_2$ production from both oxidative decarboxylation of L-[1-^{14}C]-leucine and oxidation of carbons 2-6 of L-[U-^{14}C]-leucine increased. Also, the percentage of leucine carbons 2-6 arising from oxidative decarboxylation oxidized to CO_2 did not change when skeletal muscles from fed chicks were incubated in the presence of either 0.2 or 1 mM octanoate (Table IV-1). Any increase in the release of isovalerate by skeletal muscle in the presence of octanoate (Spydevold and Hokland, 1983) may be simply due to the fact that the production of isovaleryl-CoA parallels the increased rate of decarboxylation of KIC. Since the rate of net leucine transamination is increased by octanoate in skeletal muscle from fed chicks (Table 1), increased formation of KIC increases the intracellular concentrations of KIC and also stimulates the activity of BCKA dehydrogenase (Randle et al., 1984) probably by inhibiting BCKA dehydrogenase kinase (Paxton and Harris, 1984). These overall effects may account for decreased Km values of BCKA dehydrogenase in muscle homogenates in the presence of octanoate (Paul and Adibi, 1978).

Reports in the literature on the effect of octanoate on leucine decarboxylation in skeletal muscle from fasted rats are variable (Paul and Adibi, 1978; Wagenmakers and Veerkamp, 1984). These variations may be due to differences in the experimental conditions such as use of muscle homogenates or intact whole muscle and the initial extent of activation of BCKA dehydrogenase. In cardiac muscle, for example, the effect of octanoate on leucine decarboxylation has been shown to depend upon the initial extent of activation of BCKA dehydrogenase (Buxton et al., 1984). Wagenmakers and Veerkamp (1984) reported that

octanoate increased the oxidative decarboxylation of BCKA in quarter-diaphragms from 1-day fasted rats, but had no effect in quarter-diaphragms from 3-day fasted rats. In contrast, Paul and Adibi (1978) reported that octanoate increased the rate of leucine oxidative decarboxylation in skeletal muscle homogenates from 5-day fasted rats. However, since the capacity of muscle homogenates to decarboxylate leucine is greatly reduced (Paul and Adibi, 1976), the initial activity of BCKA dehydrogenase may be lower in the muscle homogenates from 5-day fasted rats than in intact skeletal muscle preparations thus accounting for the activation by octanoate. In this study, we found that octanoate stimulated the oxidative decarboxylation of leucine in incubated skeletal muscle from fed chicks (Table IV-1) in which the activity of BCKA dehydrogenase is low but had no effect in skeletal muscles from the 24-h fasted chicks (Table 2). These results suggest that under conditions such as fasting in which BCKA dehydrogenase is activated (Odessey and Goldberg, 1979), strong activators such as octanoate may not have an additional stimulating effect. The present studies also imply that the effects of fasting and octanoate on leucine decarboxylation are not additive.

The rate of KIC release was not altered in the skeletal muscle from either the fed or the 24-h fasted chicks in the presence of octanoate (Tables IV-1,2). Spydevold and Hokland (1983) also reported that the rate of KIC release from the perfused rat hindquater was not changed in the presence of 1 mM octanoate. This is apparently due to the fact that the increase in the net rate of leucine transamination is associated with an increase in the rate of KIC decarboxylation in the presence of octanoate.

In the resting muscle from fed animals, glucose is the prefered oxidative substrate for ATP production (Rennie and Edwards, 1981). Based on the present understanding of the regulation of BCKA dehydrogenase by ATP-dependent phosphorylation and dephosphorylation (Randle et al., 1984), the addition of glucose to the muscle incubation media is expected to inhibit the oxidative decarboxylation of leucine due to an increased supply of ATP. In this study, 0, 5 and 12 mM glucose were employed to examine the effect of glucose on leucine degradation in EDC muscles from both fed and fasted chicks. As shown in

Table IV-3, the presence of 5 mM and 12 mM glucose inhibits the rate of oxidative decarboxylation of leucine in skeletal muscle from fed chicks as has been previously reported in hemidiaphragms from fed rats by Odessey and Goldberg (1972) and Buse et al. (1972). Glucose possibly inhibits leucine decarboxylation in fed chick muscles by increasing the concentration of NADH which is an inhibitor of BCKA dehydrogenase (Randle et al., 1984) and by decreasing the concentration of NAD^+ and CoA-SH concentrations which are cofactors of this enzyme (Randle et al., 1984) in addition to increasing ATP production. It is possible that glucose stimulates phosphorylation of skeletal muscle BCKA dehydrogenase as a 68KDa islet-cell protein and purified skeletal muscle phosphoglucomutase (Colca et al., 1984), but direct evidence needs to be provided. On the other hand, the addition of glucose had no inhibiting effect on the rate of oxidative decarboxylation of leucine in skeletal muscle from 24-h fasted chicks in which the activity of BCKA dehydrogenase is increased (Table IV-4). These results are in contrast to those reported by Palmer et al. (1985) in which 5 mM glucose markedly increased the rate of CO_2 production from both leucine decarboxylation and total leucine oxidation in hemidiaphragms from 40-h fasted rats. The present observations imply that under conditions such as fasting in which the initial activity of BCKA dehydrogenase is activated, inhibitors of leucine decarboxylation such as glucose may not have any effect in skeletal muscle. In addition, the results obtained in this study may in part explain why Wagenmakers and Veerkamp (1984) did not observe an effect of glucose on the rate of oxidative decarboxylation of BCKA in the rat quater diaphragms incubated in the absence of insulin and amino acids since the activity of BCKA dehydrogenase would be activated under such experimental conditions.

Our data suggest that the regulation of leucine degradation in skeletal muscle by factors such as octanoate and glucose depends on the initial activity of BCKA dehydrogenase as previously shown to occur in cardiac muscle (Buxton et al., 1984). Under conditions such as fasting in which the activity of BCKA dehydrogenase is activated, stimulation of leucine degradation by activators such as octanoate (Table IV-1) or inhibition of leucine degradation

by inhibitors such as glucose (Table IV-3) normally observed in muscles of fed animals do not appear to be effective. On the other hand, studies presented in Chapter 3 show that ketone bodies which inhibit leucine degradation in the skeletal muscle from fed chicks inhibit leucine degradation to a greater extent in EDC muscle from 24-h fasted chicks (Chapter 3). This may be due to the fact that oxidation of glucose is inhibited (Goodman, et al., 1974) but the oxidation of ketone bodies is enhanced in skeletal muscle of fasted animals (Ruderman and Goodman, 1973). When glucose oxidation is inhibited, the presence of glucose in the incubation media would not result in changes in concentrations of NAD^+, CoA-SH, NADH, ATP in skeletal muscles as compared to the absence of glucose. However, when the oxidation of ketone bodies is stimulated, the presence of ketone bodies in the incubation media would increase the concentrations of NADH and ATP, but decrease the concentrations of NAD^+ and CoA-SH. Thus it becomes apparent that glucose and ketone bodies must be metabolized before their inhibiting effect on leucine degradation in skeletal muscle occurs.

Table IV-1. The effect of octanoic acid on leucine degradation in skeletal muscle from fed chicks.

Incubation†* medium octanoate concentration	Leucine Metabolism (nmol h⁻¹ mg muscle⁻¹) CO_2 from total leucine oxidation (a)	CO_2 from leucine decarboxylation (b)	CO_2 from oxidation of leucine carbons 2-6 (c=a-b)	Net KIC Production (d)	Net Transamination (e=b+d)	Percentage of transaminated leucine released as KIC (dx100%)e⁻¹	Percentage of leucine carbons 2-6 oxidized to CO_2 (cx100%)(5b)⁻¹
0	1.40‡A ±0.03	0.41 A ±0.01	0.97 Aa ±0.03	0.13 ±0.01	0.54 Aa ±0.01	23.9 A ±1.8	47.8 ±2.0
0.2	1.81 B ±0.09	0.56 B ±0.05	1.24 ABb ±0.07	0.15 ±0.01	0.71 Bb ±0.05	22.4 A ±2.4	47.0 ±4.7
1.0	2.36 C ±0.12	0.71 C ±0.04	1.65 Bc ±0.09	0.12 ±0.01	0.83 Bc ±0.04	14.2 B ±1.4	47.2 ±2.6

† n=10 per treatment
‡ Mean ± SEM
* Incubation media contained insulin, amino acids and glucose.
a,b,c: Means within the column followed by different letters a,b,c are significantly different ($P<0.05$).
A,B,C: Means within the column followed by different letters A,B,C are significantly different ($P<0.01$).

Table IV-2. The effect of octanoic acid on leucine degradation in skeletal muscle from 24 h fasted chicks.

	Leucine Metabolism (nmol h^{-1} mg $muscle^{-1}$)						
Incubation†• medium octanoate concentration (mM)	CO_2 from total leucine oxidation (a)	CO_2 from leucine decarboxy-lation (b)	CO_2 from oxidation of leucine carbons 2-6 (c=a-b)	Net KIC Production (d)	Net Trans-amination (e=b+d)	Percentage of trans-aminated leucine released as KIC (dx100%)e^{-1}	Percentage of leucine carbons 2-6 oxidized to CO_2 (cx100%)$(5b)^{-1}$
0	2.09‡ ±0.12	0.66 ±0.03	1.43 ±0.10	0.14 ±0.01	0.80 ±0.03	**17.8** ±1.3	**43.3** ±2.9
0.2	1.98 ±0.10	0.64 ±0.01	1.34 ±0.09	0.15 ±0.01	0.78 ±0.02	**18.8** ±1.3	**41.9** ±3.2
1.0	2.23 ±0.12	0.69 ±0.02	1.54 ±0.13	0.14 ±0.01	0.83 ±0.02	**17.0** ±0.9	**44.6** ±4.5

† n=10 per treatment
‡ Mean ± SEM
• Incubation media contained insulin, amino acids and glucose.

Table IV-3. The effect of glucose on leucine degradation in skeletal muscle from fed chicks.

	Leucine Metabolism (nmol h^{-1} mg muscle $^{-1}$)						
Incubation†* medium glucose concentration (mM)	CO_2 from total leucine oxidation (a)	CO_2 from leucine decarboxy-lation (b)	CO_2 from oxidation of leucine carbons 2-6 (c=a-b)	Net KIC Production (d)	Net Trans-amination (e=b+d)	Percentage of trans-aminated leucine released as KIC (dx100%)e^{-1}	Percentage of leucine carbons 2-6 oxidized to CO_2 (cx100%)$(5b)^{-1}$
0	2.06‡A ±0.13	0.56 A ±0.03	1.50 A ±0.11	0.07 Aa ±0.01	0.63 ±0.04	10.4 A ±1.0	53.8 ±3.3
5.0	1.44 B ±0.08	0.41 B ±0.02	1.03 B ±0.07	0.12 ABb ±0.01	0.53 ±0.03	22.2 B ±1.6	51.9 ±4.9
12.0	1.35 B ±0.07	0.39 B ±0.02	0.96 B ±0.06	0.18 Bc ±0.02	0.56 ±0.02	31.3 C ±2.8	50.9 ±3.6

† n=10 per treatment
‡ *Mean ± SEM*
* Incubation media were complete except for glucose as indicated.
a,b,c : Means followed by different letters a,b,c within the column are significantly different ($P<0.05$).
A, B, C : Means followed by different letters A, B, C within the column are significantly different ($P<0.01$).

Table IV-4. The effect of glucose on leucine degradation in skeletal muscle from 24-h fasted chicks.

	Leucine Metabolism (nmol h^{-1} mg muscle $^{-1}$)						
Incubation†* medium glucose concentration (mM)	CO_2 from total leucine oxidation (a)	CO_2 from leucine decarboxy-lation (b)	CO_2 from oxidation of leucine carbons 2-6 (c=a-b)	Net KIC Production (d)	Net Trans-amination (e=b+d)	Percentage of trans-aminated leucine released as KIC (dx100%)e^{-1}	Percentage of leucine carbons 2-6 oxidized to CO_2 (cx100%)$(5b)^{-1}$
0	1.75‡ ±0.06	0.58 ±0.02	1.17 ±0.05	0.14 ±0.02	0.72 ±0.04	**19.6 ±1.7**	**40.5 ±1.9**
5.0	1.71 ±0.07	0.57 ±0.03	1.14 ±0.05	0.14 ±0.01	0.71 ±0.03	**19.9 ±2.3**	**40.9 ±2.3**
12.0	1.71 ±0.05	0.55 ±0.02	1.16 ±0.05	0.15 ±0.02	0.70 ±0.02	**21.4 ±2.2**	**42.8 ±2.6**

† n = 10 per treatment
‡ Mean ± SEM
* Incubation media were complete except for glucose as indicated.

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V. Arachidonic Acid, Prostaglandin E_2 and $PGF_{2\alpha}$ Inhibit Leucine Degradation in Chick Skeletal Muscle

A. Introduction

Leucine is extensively catabolised by skeletal muscle (Odessey and Goldberg, 1972). The degradation of leucine is initiated by a reversible transamination to α-ketoisocaproate (KIC) catalysed by BCAA aminotransferase, followed by irreversible decarboxylation of KIC to isovaleryl-CoA catalysed by branched-chain α-ketoacid (BCKA) dehydrogenase. BCKA dehydrogenase is subject to regulation by an ATP-dependent phosphorylation and dephosphorylation cycle in skeletal muscle, liver, heart and kidney (Randle et al., 1984; Paxton et al., 1986). This enzyme complex is inactivated by BCKA dehydrogenase kinase, and activated by BCKA dehydrogenase phosphatase (Randle et al., 1984).

It is well established that prostaglandins participate in a variety of physiological and pathological processes. For example, PGE_2 and $PGF_{2\alpha}$ play a role in regulating protein turnover in skeletal muscle (Rodemann and Goldberg, 1982; Smith et al., 1983). A decrease in $PGF_{2\alpha}$ release has been shown to be associated with inhibition of muscle protein synthesis by dexamethasone (Reeds and Palmer, 1984) while an increase in $PGF_{2\alpha}$ release has been shown to be associated with the stimulation of muscle protein synthesis by insulin *in vitro* (Reeds and Palmer, 1983) and *in vivo* (Reeds et al., 1985). PGE_2 has been shown to be involved in the mediation of the stimulating effect of interleukin-1 on muscle protein degradation (Baracos et al., 1983). In addition, both PGE_2 and PGE_1 have been demonstrated to increase the sensitivity of glycolysis to insulin in rat soleus muscle (Leighton et al., 1985), enhance hepatic gluconeogenesis (Sacca et al., 1974) and stimulate glucose oxidation in rat (Chang and Roth, 1981) and human (Richelsen et al., 1985) adipocytes.

Although prostaglandins have been shown to be involved in the regulation of a variety of biochemical processes there is virtually no information concerning their involvement in the control of amino acid metabolism in animal tissues. The purpose of this present study was to investigate whether arachidonic acid, PGE_2 and $PGF_{2\alpha}$ can influence leucine catabolism in

chick skeletal muscle *in vitro*.

B. Materials and Methods

L-[U-^{14}C]leucine and L-[1-^{14}C]leucine were purchased from ICN Radiochemicals, Montreal, Que. Radiochemical purity was determined to be greater than 99% by descending paper chromatography using n-butanol:acetic acid:H_2O (2:1:1) as the solvent. Arachidonic acid, PGE_2 and $PGF_2\alpha$, indomethacin (an inhibitor of prostaglandin synthesis) and other chemicals used were purchased from Sigma Chemical Co., St. Louis, MO, USA. Rabbit anti-PGE_2 was obtained from Miles Laboratories, Naperville, IL. The cross-activity of anti-PGE_2 with PGE_1 and $PGF_2\alpha$ was 136% and 22%, respectively, according to the manufacturer.

Male broiler chicks weighing 125-140 g, which had been allowed free access to food and water were used. The extensor digitorum communis (EDC) muscles were dissected and preincubated for 30 min as described in Chapter 2. The left and right EDC muscles were then transferred to fresh preincubation media containing L-[U-^{14}C]-leucine and L-[1-^{14}C]-leucine, respectively for a final incubation of 2 h. Arachidonic acid, indomethacin, PGE_2 and $PGF_2\alpha$ were present in both the preincubation and final incubation media at the initial concentrations noted in Tables V-1, -2 and -3. At the end of each 2 h final incubation, collection of $^{14}CO_2$ produced from the oxidative decarboxylation of L-[1-^{14}C]leucine, total oxidation of L-[U-^{14}C]leucine and decarboxylation of [1-^{14}C]KIC was performed as described in Chapter 2. Linearity of $^{14}CO_2$ production over the 2 h incubation period and over the muscle weight used has been established previously (Chapter 2).

Calculations of leucine decarboxylation rate, net rate of leucine transamination and net rate of KIC release were performed as described by Aftring et al. (1985). The rates of CO_2 production from the total oxidation of leucine, the percentage of decarboxylated leucine oxidised to CO_2 and the percentage of transaminated leucine released as KIC were calculated as described by Wijayasinghe et al. (1983). The rate of CO_2 production from oxidation of

carbons 2-6 of decarboxylated leucine was calculated as the rate of CO_2 production from the total oxidation of leucine minus the leucine decarboxylation rate.

For determination of PGE_2 production by skeletal muscle, 25 ten-day-old broiler chicks were used with 5 birds for each treatment. The left and right EDC muscles from the same chick were inserted into stainless steel supports, dissected and preincubated together for 30 min as described in Chapter 2. The EDC muscles were then transferred to fresh incubation media for a 2 h final incubation. At the end of the final incubation, the muscles were removed and the incubation media were immediately stored at -70°C until analysis. A 0.2 ml portion of the incubation media was used without extraction to measure the release of PGE_2 by chick skeletal muscle as described by Jaffe and Behrman (1974).

The results were statistically analysed by the procedures of one-way variance analysis and the SNK multiple comparison test using the pooled error term as described by Steel & Torrie (1980).

C. Results

The Effect of Arachidonic Acid and Indomethacin on Leucine Degradation in Chick Muscle

The effect of arachidonic acid on leucine degradation in skeletal muscle of fed chicks is shown in Table V-1. Arachidonic acid (5 μM) inhibited the net rate of transamination of leucine by 18.9% ($P<0.05$) and the rate of leucine oxidative decarboxylation by 22.5% ($P<0.01$). This agent also decreased the rates of CO_2 production from total leucine oxidation ($P<0.01$) and from the oxidation of leucine carbons 2-6 ($P<0.05$). Arachidonic acid did not influence the production of KIC, the percentage of transaminated leucine released as KIC or the percentage of carbons 2-6 of decarboxylated leucine oxidised to CO_2.

Indomethacin (50 μM) had no effect on the basal rates of leucine degradation in chick skeletal muscle (Table V-1). Although indomethacin at 5 μM did not prevent the inhibition of the net rate of leucine transamination by arachidonic acid, indomethacin at 50 μM completely

reversed the arachidonic acid-induced decrease of the net rate of leucine transamination to control values (Table V-1). This inhibitor of prostaglandin synthesis at both 5 μM and 50 μM prevented the inhibiting effect of arachidonic acid on the rates of leucine oxidative decarboxylation ($P<0.01$), and the rates of CO_2 production from the total oxidation of leucine ($P<0.01$). At concentrations of 5 μM and 50 μM, indomethacin also prevented the inhibiting effect of arachidonic acid on the rates of CO_2 production from the oxidation of leucine carbons 2-6 at $P<0.05$ and $P<0.01$, respectivly.

The Effect of Prostaglandin E_2 and $F_2\alpha$ on Leucine Degradation in Chick Muscle

The effect of PGE_2 on leucine degradation in chick skeletal muscle is presented in Table V-2. PGE_2 at concentrations as low as 0.28 μM inhibited the net rate of leucine transamination by 20% ($P<0.01$) and the rate of oxidative decarboxylation of leucine by 16.7% ($P<0.05$). PGE_2 at 0.28 μM also inhibited ($P<0.01$) the rates of CO_2 production from the total oxidation of leucine and the oxidation of carbons 2-6 of the decarboxylated leucine by 21.9% and 24.0%, respectively. Higher concentrations of PGE_2 (0.7-2.8 μM) did not further inhibit the rates of leucine degradation in the EDC muscle. PGE_2 at all concentrations studied did not influence the rate of KIC release, the percentage of transaminated leucine as KIC released or the percentage of leucine carbons 2-6 oxidised to CO_2.

$PGF_2\alpha$ at 2.8 μM had no effect on the rates of leucine degradation in chick skeletal muscle (Table V-3). However, 14 μM $PGF_2\alpha$ in the incubation medium significantly inhibited the rates of CO_2 production from total leucine oxidation ($P<0.01$) and the rates of CO_2 production from the oxidation of leucine carbons 2-6 ($P<0.01$). $PGF_2\alpha$ at 14 μM also inhibited the rates of leucine oxidative decarboxylation ($P<0.01$) and the net rates of leucine transamination ($P<0.01$). Like PGE_2, $PGF_2\alpha$ did not affect the net rates of KIC release, the percentage of transaminated leucine released as KIC or the percentage of leucine carbons 2-6 oxidised to CO_2 (Table 2).

Apparent Production of Prostaglandin E_2 by the Chick Muscle

The values for the rate of prostaglandin E_2 production by chick EDC muscle are presented in Table V-4. This rate is refered to as apparent rate since prostaglandin E_2 is degraded due to its unstability in aqueous solution (Stehle, 1982). Incubation in the presence of 50 μM indomethacin reduced the rate of basal production of PGE_2 to undetectable levels. Arachidonic acid (5 μM) increased ($P<0.01$) the production of PGE_2 13.6-fold above the rate measured in the control muscles. Indomethacin at 5 μM inhibited ($P<0.01$) the production of PGE_2 in the presence of the added arachidonic acid by 41%. This drug at 50 μM further inhibited the production of PGE_2 by 73.5% in the presence of 5 μM arachidonic acid.

D. Discussion

The present study reveals that arachidonic acid, PGE_2 and $PGF_2\alpha$ may play a role in the regulation of leucine catabolism in skeletal muscle. Arachidonic acid at a concentration of 5 μM, which has been shown to stimulate protein degradation in rat skeletal muscle (Rodemann and Goldberg, 1982), inhibited the rates of leucine degradation in the chick EDC muscle (Table V-1). The inhibition of leucine degradation by arachidonic acid may be due to one or more of its metabolites which include the prostaglandins, and leukotrienes. If the inhibition of leucine catabolism by arachidonic acid is due to increased production of prostaglandins, this effect should be blocked by indomethacin. The present results show that indomethacin at 5 μM prevented the inhibition by arachidonic acid of the rates of leucine oxidative decarboxylation, CO_2 production from total leucine oxidation and the oxidation of leucine carbons 2-6. Indomethacin at 50 μM, which has been used by Smith et al.(1983) in studies of the effect of arachidonic acid on protein turnover in rabbit skeletal muscle, completely blocked the inhibiting effect of arachidonic acid on leucine degradation in chick EDC muscle (Table V-1). Therefore, since indomethacin itself did not have a effect on leucine degradation (Table V-1), the actions of arachidonic acid on leucine degradation in chick skeletal muscle appears to be mediated by metabolites generated via the cyclooxygenase

pathway.

In order to determine the response of prostaglandin synthesis in chick skeletal muscle to arachidonic acid and indomethacin, we measured the release of PGE_2 as an index of prostaglandin production from chick EDC muscle. In the presence of 5 μM arachidonic acid, indomethacin at 5 μM and 50 μM inhibited the release of PGE_2 by 41% and 73.5%, respectively (Table V-4). Although the antibody used for the detection of PGE_2 in this study cross-reacts with PGE_1, the dramatic decrease in the release of antigen in the presence of both arachidonic acid and indomethacin reflects the actual inhibition of PGE_2 production since arachidonic acid is the precursor of "2 series" prostaglandins such as PGE_2, but not precursor of "1 series" prostaglandins such as PGE_1 (Samuelsson et al., 1978). Thus, it is clear that there is a close link between the production of prostaglandin E_2 (Table V-4) and the inhibiting effect of arachidonic acid on leucine degradation (Table V-1).

It is not clear how PGE_2 and $PGF_2\alpha$ inhibit leucine degradation in chick skeletal muscle. The actions of prostaglandins on the cellular metabolism appear to result from their specific binding to the cell membrane, but very little information is available regarding the immediate post-receptor consequences (Robertson, 1986). Because incubation medium PGE_2 concentrations greater than 0.28 μM did not have a further inhibiting effect on leucine degradation (Table V-1), it is likely that the binding of PGE_2 to the receptors on the plasma membrane may become saturated as the concentrations of PGE_2 increases. The second messenger(s) produced from the binding of the PGE_2 and $PGF_2\alpha$ to their receptors on the plasma membrane may trigger a series of reactions, resulting in inhibition of either BCAA aminotransferase or BCKA dehydrogenase or both.

The fact that $PGF_2\alpha$ at 2.8 μM failed to inhibit leucine degradation in the chick skeletal muscle suggests that PGE_2 is more potent than $PGF_2\alpha$ in the control of leucine degradation in the chick skeletal muscle. It may be argued that the PGE_2 and $PGF_2\alpha$ added may not be at their physiological concentrations and thus the observed effects on leucine degradation may not be of physiological significance. However, since PGE_2 and $PGF_2\alpha$ are

not stable in aqueous solutions at 37°C and pH 7.4 (Stehle, 1982), the actual amount of these two agents in the incubation media must be lower than that calculated for the start of the incubation. Smith et al. (1983) have shown that $PGF_2\alpha$ at 2.8 μM stimulated protein synthesis in rabbit skeletal muscle *in vitro* and Reeds at al.(1985) have shown that $PGF_2\alpha$ mediated the effect of insulin on muscle protein synthesis in rats *in vivo*. Thus, our present *in vitro* studies may have relevence to the understanding of *in vivo* metabolism of leucine in skeletal muscle.

It is interesting to note that some of the factors which can influence the production of prostaglandins have been shown to regulate leucine degradation in skeletal muscle. For example, starvation, which reduces $PGF_2\alpha$ release by rabbit skeletal muscle (Smith et al. 1983), has been shown to increase leucine degradation in skeletal muscle from rats (Goldberg and Odessey, 1972) and chicks (Chapter 2). Furthermore, insulin, which increases the production of PGE_2 and $PGF_2\alpha$ by skeletal muscles of rabbits *in vitro* (Reeds and Palmer, 1983) and of postabsorptive rats *in vivo* (Reeds et al., 1985), has been demonstrated to inhibit the rates of leucine transamination and decarboxylation in the perfused hindquarter of fed rats (Hutson et al., 1978; 1980) and the rate of leucine decarboxylation in skeletal muscle from fed chicks (our unpublished observations). It remains to be determined whether fasting and insulin influence leucine degradation via changes in prostaglandin production.

Table V-1. The effect of arachidonic acid on leucine degradation in skeletal muscle from fed chicks.

	Leucine Metabolism (nmol h^{-1} mg muscle^{-1})						
Treatment†	CO_2 from total leucine oxidation (a)	CO_2 from leucine decarboxy-lation (b)	CO_2 from oxidation of leucine carbons 2-6 (c=a-b)	Net KIC Production (d)	Net Trans-amination (e=b+d)	Percentage of trans-aminated leucine released as KIC (dx100%)e^{-1}	Percentage of leucine carbons 2-6 oxidized to CO_2 (cx100%)(5b)$^{-1}$
Control	1.42 ‡A ±0.08	0.40 A ±0.02	1.02 A ±0.06	0.12 ±0.01	0.53 A ±0.03	23.9 ab ±1.4	51.2 ±2.5
50 μM IN	1.56 A ±0.06	0.43 A ±0.02	1.14 A ±0.05	0.11 ±0.01	0.54 A ±0.02	20.9 a ±1.6	53.7 ±3.0
5 μM AA	1.13 B ±0.05	0.31 B ±0.02	0.81 B ±0.04	0.12 ±0.01	0.43 B ±0.02	27.4 b ±2.5	53.3 ±3.9
5 μM AA +5 μM IN	1.41 A ±0.05	0.38 AB ±0.01	1.[illegible] ±0.05	0.11 ±0.01	0.49 AB ±0.02	22.0 ab ±1.9	54.2 ±3.3
5 μM AA +50 μM IN	1.[illegible]4 A ±0.06	0.44 A ±0.01	1.20 A ±0.05	0.11 ±0.01	0.54 A ±0.02	19.[illegible] a ±[illegible]	54.4 ±2.3

† n = 10 per treatment
‡ Mean ± SEM
AA: arachidonic acid; IN: indomethacin.
a, b: Means followed by different letters a, b within the column are significantly different ($P<0.05$).
A, B: Means followed by different letters A, B within the column are significantly different ($P<0.01$)

Table V-2. The effect of prostaglandin E_2 on leucine degradation in skeletal muscle from fed chicks.

	Leucine Metabolism (nmol h^{-1} mg muscle^{-1})						
PGE_2† concentration (μM)	CO_2 from total leucine oxidation (a)	CO_2 from leucine decarboxylation (b)	CO_2 from oxidation of leucine carbons 2-6 (c=a-b)	Net KIC Production (d)	Net Transamination (e=b+d)	Percentage of transaminated leucine released as KIC (dx100%)e^{-1}	Percentage of leucine carbons 2-6 oxidized to CO_2 (cx100%)(5b)$^{-1}$
0	1.46 ‡Aa ±0.05	0.42 Aa ±0.01	1.04 A ±0.05	0.12 ±0.01	0.55 A ±0.02	21.9 ±1.8	49.0 ±2.8
0.14	1.29 ABab ±0.04	0.38 ABab ±0.02	0.91 AB ±0.03	0.09 ±0.01	0.47 AB ±0.02	19.1 ±2.2	47.9 ±2.6
0.28	1.14 Bbc ±0.05	0.35 ABb ±0.02	0.79 B ±0.05	0.09 ±0.01	0.44 B ±0.02	19.7 ±2.1	46.3 ±3.5
0.70	1.13 Bb ±0.04	0.35 ABb ±0.01	0.78 B ±0.04	0.10 ±0.01	0.45 B ±0.02	19.0 ±3.4	50.64.2 ±3.1
1.40	1.14 Bbc ±0.02	0.34 Bb ±0.01	0.79 B ±0.03	0.08 ±0.01	0.42 B ±0.01	19.0 a ±1.9	50.6 ±3.0
2.80	1.09 Bc ±0.04	0.31 Bb ±0.02	0.77 B ±0.04	0.08 ±0.01	0.39 B ±0.02	18.3 ±1.8	50.6 ±4.2

† n=10 per treatment
‡ Mean ± SEM
a,b,c: Means followed by different letters a, b, c within the column are significantly different ($P<0.05$).
A, B: Means followed by different letters A, B within the column are significantly different ($P<0.01$).

Table V-3. The effect of prostaglandin $F_2\alpha$ on leucine degradation in skeletal muscle from fed chicks.

	Leucine Metabolism (nmol h^{-1} mg muscle^{-1})						
$PGF_2\alpha$† concentration (μM)	CO_2 from total leucine oxidation (a)	CO_2 from leucine decarboxy-lation (b)	CO_2 from oxidation of leucine carbons 2-6 (c=a-b)	Net KIC Production (d)	Net Trans-amination (e=b+d)	Percentage of trans-aminated leucine released as KIC (dx100%)e	Percentage of leucine carbons 2-6 oxidized to CO_2 (cx100%)(5b)$^{-1}$
0	1.48 ‡A ±0.07	0.45 A ±0.01	1.03 A ±0.06	0.11 ±0.01	0.56 A ±0.03	19.2 ±1.2	45.8 ±2.4
0.28	1.41 A ±0.07	0.45 A ±0.01	0.96 A ±0.06	0.10 ±0.01	0.55 A ±0.02	18.4 ±1.7	42.9 ±3.5
14.0	0.99 B ±0.06	0.33 B ±0.02	0.68 B ±0.06	0.10 ±0.01	0.43 B ±0.02	23.2 ±1.5	41.5 ±3.3

† n=10 per treatment
‡ Mean ± SEM
A, B Means followed by different letters A, B within the column are significantly different ($P<0.01$).

Table V-4. Prostaglandin E_2 production by chick [illegible]C muscle

Treatment †	PGE_2 production (pg $2h^{-1}$ mg muscle $^{-1}$)
Control	15.9 ± 0.7 ‡ A
50 μM IN	ND
5 μM AA	232.9 ± 7.3 B
5 μM AA + 5 μM IN	136.6 ± 8.9 C
5 μM AA + 50 μM IN	61.8 ± 3.9 D

†=5 per treatment.
‡ Mean ± SEM.
ND: not detectable.
IN: indomethacin.
AA: arachidonic acid
A-D: Means within the column followed by different letters A-D are significantly different ($P<0.01$).

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VI. General Discussion and Conclusions

Isolated intact skeletal muscle preparations have been widely used in *in vitro* studies of leucine degradation (e.g. Odessey and Goldberg, 1972; Aftring et al., 1985) The use of these preparations avoids the greatly reduced rate of leucine oxidative decarboxylation and the loss of oxidation of leucine carbons 2-6 (Paul and Adibi, 1976) found with muscle homogenates. In addition, the muscle preparation is free of interference from other tissues in the body. In the present study, intact EDC muscles from chick wings were used for studying leucine degradation in chick skeletal muscle. This muscle preparation has been shown to be valid for in vitro studies of protein turnover as measured by linear increase in the release of all twenty amino acids during incubation and in the constant levels of ATP, phosphocreatine, glycogen and prostaglandin E_2 (Baracos and Langman, unpublished observations). In this study, muscles were incubated in the presence of 0.5 mM ^{14}C-leucine to facilitate the specific radioactivity of intracellular leucine to rapidly reach a plateau. The net rates of transamination, leucine oxidative decarboxylation and CO_2 production from total leucine oxidation were linear from 15 min up to 2 h during the incubation period, indicating that the intracellular specific activity of leucine rapidly reached a plateau and remained constant throughout the incubation period. The amount of leucine metabolized in muscles weighing between 16 and 30 mg increased linearly with increasing muscle weight (Chapter 2). These results indicate that the uptake of amino acids by skeletal muscle fibers may not be limiting in studies using intact skeletal muscle preparation. Because plateau intracellular specific activity of leucine is lower than the specific activity of leucine in the initial incubation media (Aftring et al., 1985), the values obtained in the present study may be slight underestimations of what is occuring within the [illegible] all the calculated values may be underestimated to [illegible] extent.

Although there have been a number of studies on leucine degradation in skeletal muscle preparations from mammalian species, there is virtually no information available on branched-chain amino acid metabolism in skeletal muscle from avian species. A limited

number of studies have sho[illegible] differences in the response of leucine degradation in skeletal muscle to dietary [illegible]tion. For example, the rate of leucine oxidative decarboxylation is increased [illegible]al muscle from 3-day fasted rats (Goldberg and Odessey, 1972), but decreased in [illegible]tal fiber bundle preparations from 5-day fasted sheep (Wijayasinghe et al., 19[illegible]. Another example of a species difference in leucine degradation in skeletal muscle is that octanoate has been shown to stimulate the rates of oxidative decarboxylation of α-ketoisocaproate (KIC) and α-ketoisovalerate (KIV) in rat h[illegible]diaphragms and intact soleus muscle but inhibits these rates in human gluteus muscle fibers and pectoralis muscle fibers (Wagenmakers and Veerkamp, 1984a). A stimulating effect of octanoate on the rates of leucine oxidative decarboxylation in EDC muscle from fed chicks has been observed as shown in Table IV-1 (Chapter 4). Although information is lacking concerning the effects of ketone bodies and glucose on leucine degradation in species other than rats, ketone bodies and glucose inhibit leucine degradation in skeletal muscles from fed rats (Wagenmakers and Veerkamp, 1984 b; Odessey and Goldberg, 1972) and chicks (Tables III 2-6, Table IV-3). Thus, it appears from the results presented in this thesis that chick skeletal muscle responds to fasting and elevated concentrations of ketone bodies, octanoate and glucose in an analogous manner to that reported for intact rat skeletal muscles.

It is well documented that fasting increases the rate of leucine oxidative decarboxylation in skeletal muscles from both rats (Goldberg and Odessey, 1972; Aftring, et al., 1985) and chicks (Table II-2, Chapter 2). The mechanisms involved, however, are not understood. Based on their observations that acetoacetate stimulated leucine oxidative decarboxylation in skeletal muscle homogenates from both fed and fasted rats, Paul and Adibi (1978) suggested that increased leucine degradation in skeletal muscle during fasting may be due to increased tissue concentrations of ketone bodies. This view seems to be supported by recent observations that acetoacetyl-CoA, which is produced from metabolism of acetoacetate in skeletal muscle (Robinson and Williamson, 1980), inhibits isolated BCKA dehydrogenase kinase (Paxton and Harris (1984). In contrast to these reports, the present studies show that

both DL-β-hydroxybutyrate and acetoacetate at concentrations found in fasted chicks markedly inhibit the rates of leucine oxidative decarboxylation in EDC muscles from both fed and 24-h fasted chicks (Chapter 3). Therefore, it is not likely that increased leucine degradation in skeletal muscles of rats and chicks during fasting is related to increased plasma and tissue concentrations of ketone bodies as suggested by Paul and Adibi (1978). The possible mechaniams for increased leucine degradation during fasting may be due to : (1) increased activity of BCAA aminotransferase (Adibi et al., 1975) and/or BCKA dehydrogenase (Odessey and Goldberg, 1979); (2) increased concentrations of BCAA in skeletal muscle (Appendix 2) since Aftring et al. (1986) have recently shown that leucine and isoleucine injected into rats to achieve their concentrations within physiological ranges activate BCKA dehydrogenase; (3) elevated plasma concentrations of free long chain and medium chain fatty acids since they have been shown to stimulate leucine decarboxylation in intact skeletal muscle from fed rats (Buse et al. 1972); (4) inhibition of BCKA dehydrogenase kinase.

The present studies demonstrate that the regulation of leucine degradation in chick skeletal muscle by oxidative substrates such as ketone bodies, octanoate and glucose is complex. DL-β-hydroxybutyrate stimulates the net rate of leucine transamination in skeletal muscle from fed chicks but acetoacetate has no effect (Tables 1, 2-3, Chapter 3). These observations suggest that in skeletal muscle from fed chicks, increased NADH concentrations may increase the net rate of transamination of leucine. However, in skeletal muscle from 24-h fasted chicks, both DL-β-hydroxybutyrate and acetoacetate inhibit the net rate of leucine transamination (Chapter 3), probably due to inhibition of BCAA aminotransferase by metabolites of ketone bodies but not likely due to a decrease in intracellular specific activity of leucine based on the following observations: (1) ketone bodies inhibit the net rate of protein degradation in skeletal muscle from fasted chicks (Appendix 3); (2) ketone bodies do not affect the rate of protein synthesis in skeletal muscle (Fulks et al., 1976); (3) ketone bodies do not influence the uptake of leucine by skeletal muscle from fasted chicks (our

unpublished observations). The inhibition of the net rate of leucine transamination during fasting may in part account for decreased release of alanine and glutamine as reported by Palaiologos and Felip (1978). Ketone bodies which inhibit the rate of leucine oxidative decarboxylation in EDC muscle from fed chicks inhibit this rate in EDC muscle from 24-h fasted chicks to an even greater extent (Table III 2-3). This inhibition of leucine oxidative decarboxylation by ketone bodies in skeletal muscle from both fed and fasted chicks is independent of leucine uptake, and insulin, glucose and amino acid concentrations (Chapter 3). Further inhibition of this rate by ketone bodies in skeletal muscle from fasted chicks may be due to the fact that ketone bodies undergo more extensive oxidation in skeletal muscle of fasted animals than fed animals (Ruderman and Goodman, 1973), thus resulting in a greater depletion of NAD^+ and CoA-SH, which are cofactors of BCKA dehydrogenase (Randle et al., 1984) and higher concentrations of NADH, which is an inhibitor of BCKA dehydrogenase (Randle et al., 1984). It is also likely that increased oxidation of ketone bodies increases ATP production, thus inhibiting BCKA dehydrogenase. The inhibition of leucine decarboxylation in skeletal muscle preparations by ketone bodies is consistent with observations made *in vivo* indicating that there is an inverse relationship between plasma concentrations of ketone bodies and whole body rates of leucine decarboxylation (Beaufrere et al., 1975; Tessari et al., 1986). Our present observations may also help, in part, explain why nitrogen retention is improved when animals are fed high-fat diets (Reeds et al., 1981).

To test whether activators and inhibitors of leucine degradation in skeletal muscle in which the activity of BCKA dehydrogenase is low may have different effects in muscles in which the activity of BCKA dehydrogenase is relatively higher, we chose octanoate as an example of an activator and glucose as an example of an inhibitor. Glucose has no effect on the net rate of leucine transamination but inhibits leucine oxidative decarboxylation in skeletal muscle probably by increasing ATP and decreasing CoA-SH and NAD^+ concentrations. However, glucose has none of these effects in EDC muscle from 24-h fasted chicks. This may be due to the fact that glucose oxidation is inhibited in skeletal muscle from fasted animals

(Goodman et al., 1974; Hagg, et al., 1976), resulting in minimal change in the intracellular concentrations of NAD$^+$ and CoA-SH. In contrast to glucose, octanoate markedly stimulates the net rates of leucine transamination and leucine oxidative decarboxylation in EDC muscles from fed chicks but has no such effects in EDC muscle from 24-h fasted chicks (Tables IV 1-2, Chapter 4). The stimulation of leucine decarboxylation by octanoate may be due to direct inhibition of BCKA dehydrogenase kinase (Paxton and Harris, 1984) when the initial activity of BCKA dehydrogenase is low. Odessey and Goldberg (1979) have suggested that the increased activity of BCKA dehydrogenase during fasting may be due to inhibition of BCKA dehydrogenase kinase. If this is the case, the lack of an effect of octanoate on leucine decarboxylation in skeletal muscle from fasted chicks may be due to a lack of further inhibition of this kinase by octanoate during fasting. These studies indicate that the effects of some compounds on leucine degradation in skeletal muscle depend on the initial activity of BCKA dehydrogenase.

It should be pointed out that leucine degradation in skeletal muscle from both fed and fasted chicks responds to ketone bodies, octanoate and glucose in different manners. This difference may reflect different mechanisms whereby these oxidative substrates regulate metabolism of this essential amino acid in skeletal muscle. Future studies are needed to test whether ketone bodies and glucose inhibit leucine oxidative decarboxylation in skeletal muscle through increased ATP production and/or depletion of BCKA dehydrogenase cofactors such as NAD$^+$ and CoA-SH.

Although prostaglandins have been shown to be involved in the regulation of protein turnover (Rodeman and Goldberg, 1982; Smith et al., 1983) and increased sensitivity of glycolysis to insulin (Leighton et al., 1985) in skeletal muscle, there has been no information published concerning the effect of these compounds on amino acid metabolism in animal tissues. The present studies show that PGE_2 and $PGF_{2\alpha}$ inhibit the net rates of leucine transamination, leucine oxidative decarboxylation and CO_2 production from both total leucine oxidation and oxidation of leucine carbons 2-6 in EDC muscle from fed chicks (Table V 2-3,

Chapter 5). It is interesting to note that insulin, which increases production of PGE_2 and $PGF_{2\alpha}$ by rat skeletal muscle (Reeds and Palmer, 1983; Reeds et al., 1985), has been shown to inhibit the rate of leucine oxidative decarboxylation in perfused hindquarter of fed rats (Hutson, et al., 1978; 1980) and EDC muscle of fed chicks (our unpublished observations). Since insulin appears to act on muscle protein turnover through prostaglandin production (Reeds and Palmer, 1983; Reeds et al., 1985), it is possible that this hormone acts through prostaglandin production to inhibit leucine metabolism. This hypothesis needs to be tested in future studies.

From the observations presented in this thesis, the following conclusions can be drawn:

(1) chick EDC muscle appears to be suitable for *in vitro* studies of leucine degradation;

(2) fasting increases leucine degradation in chick skeletal muscle as previously shown in the rat hemidiaphragm, but not in sheep intercostal fiber bundle preparations;

(3) intact chick skeletal muscle responds to octanoate , ketone bodies and glucose in a manner similar to intact rat skeletal muscle incubated in the presence of insulin and amino acids but not to homogenates of rat skeletal muscles.

(4) increased concentrations of ketone bodies inhibit leucine degradation in skeletal muscle from both fed and 24-h fasted chicks;

(5) increased concentrations of ketone bodies are not responsible for the increased rate of leucine degradation in skeletal muscle during fasting;

(6) octanoate stimulates the rate of leucine degradation in EDC muscle from fed chicks but has no effect in muscle from 24-h fasted chicks;

(7) glucose inhibits the the rates of leucine oxidative decarboxylation and CO_2 production from total leucine oxidation and the oxidation of leucine carbons 2-6, but has no such effect in muscle from 24-h fasted chicks;

(8) prostaglandin E_2 and $F_2\alpha$ inhibit the net rates of leucine decarboxylation, leucine oxidative decarboxylation and CO_2 production from total leucine oxidation and the oxidation of leucine carbons 2-6 in EDC muscle from fed chicks.

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VII. Appendices

Appendix 1. Amino acid compositions in chick EDC muscle incubation medium

Amino Acid	mM
glu	0.20
ala	0.70
thr	0.60
ser	0.60
val	0.50
ile	0.25
leu	0.30
lys	0.30
his	0.20
arg	0.34
gly	0.87
asn	0.03
asp	0.03
sys	0.03
gln	0.15
met	0.10
orn	0.07
phe	0.40
pro	0.33
try	0.06
ans	0.02
tyr	0.04
aib	0.02
cit	0.04

Appendix 2. Effect of short-term fasting on free amino acid concentrations in skeletal muscle of 10-day-old broiler chicks.

Amino acid †	Fed	Fast 12 h	Fast 24 h
asp	0.88 ± 0.28	± 0.17 B	0.77 ± 0.18 A
glu	2.59 ± 0.12 A	1.66 ± 0.09 B	1.70 ± 0.02 B
asn	0.41 ± 0.07	0.53 ± 0.03	0.56 ± 0.03
ser	1.81 ± 0.26	1.95 ± 0.09	1.98 ± 0.09
gln	6.83 ± 0.61 A	3.72 ± 0.34 B	3.22 ± 0.27 B
gly	1.57 ± 0.34 A	2.74 ± 0.16 B	2.54 ± 0.10 B
thr	0.99 ± 0.15 A	1.95 ± 0.13 B	2.25 ± 0.13 B
arg	0.94 ± 0.08 A	0.64 ± 0.05 B	0.54 ± 0.02 B
tau	6.37 ± 0.41	6.74 ± 0.45	7.69 ± 0.24
ala	2.35 ± 0.13	2.32 ± 0.09	2.12 ± 0.11
tyr	0.18 ± 0.01 a	0.26 ± 0.02 b	0.25 ± 0.01 b
try	0.08 ± 0.01	0.10 ± 0.01	0.09 ± 0.01
met	0.07 ± 0.01 A	0.13 ± 0.01 B	0.14 ± 0.01 B
val	0.39 ± 0.03 a	0.46 ± 0.02 ab	0.51 ± 0.02 bc
phe	0.64 ± 0.14 A	0.25 ± 0.02 A	0.25 ± 0.01 B
ile	0.17 ± 0.01 Aa	0.21 ± 0.01 ABb	0.25 ± 0.01Bc
leu	0.27 ± 0.02 A	0.36 ± 0.02 Ba	0.44 ± 0.02 Bb
lys	1.12 ± 0.24 A	2.18 ± 0.29 B	1.98 ± 0.20 B

†The EDC muscle was homogenized with 2% TCA and then centrifuged. The supernatent was analysed for amino acids by HPLC after pre-column derivatization with OPA reagent.
‡ Values are expressed as μmol g muscle^{-1} and given as mean ± SEM, n=10 per treatment.
a-c: Means within rows followed by different letters a-c are significantly different ($P<0.05$).
A-C: Means within rows followed by different letters A-C are significantly different ($P<0.01$).

Appendix 3. Effect of fasting and ketone bodies on net protein degradation in 10-day-old broiler chick skeletal muscle.

Treatment †•	Incubation condition	Tyrosine release (pmol mg muscle $^{-1}$ 2h $^{-1}$)
Fed	none	11.45 ± 1.19 ‡ A
Fed	+ 4 mM HB	12.53 ± 0.73 A
Fed	+ 1 mM AcAc	11.36 ± 0.91 A
Fast 24 h	none	20.72 ± 2.10 Ba
Fast 24 h	+ 4 mM HB	15.12 ± 1.91 ABb
Fast 24 h	+1 mM AcAc	14.70 ± 1.21 ABb

† n=8 per treatment
‡ Mean ± SEM
• The isolated EDC muscles were incubated for 2 h in Krebs-Ringer bicarbonate buffer without insulin, amino acids or glucose.
a,b: Treatment means within the column followed by different letters a,b are significantly different ($P<0.05$).
A,B: Treatment means within the column followed by different letters A,B are significantly different ($P<0.01$).