British Journal of Nutrition (1983), **50**, 129–139

The effect of high-protein and high-carbohydrate diets on [^{125}I]iodoinsulin binding in skeletal muscle plasma membranes and isolated hepatocytes of rainbow trout (*Salmo gairdneri*)*†

BY RICHARD F. ABLETT‡, MATTHEW J. TAYLOR‖ AND DANIEL P. SELIVONCHICK§

Department of Food Science and Technology, Oregon State University, Corvallis, Oregon 97331, USA

(*Received 19 January 1983 – Accepted 9 February 1983*)

1. [^{125}I]iodoinsulin-binding studies in the presence of a concentration range of bovine insulin were conducted to establish specific insulin-binding levels in skeletal muscle plasma membranes and isolated hepatocytes of rainbow trout (*Salmo gairdneri*) reared on control, high-protein or high-carbohydrate diets.

2. Negative co-operativity was observed and receptor concentrations and apparent dissociation constants established for each preparation.

3. No differences of specific binding attributed to diet were detected in skeletal muscle plasma membrane preparations; however, the receptor concentration of isolated hepatocytes from high-carbohydrate-reared trout was increased. This contrasted to comparable mammalian studies.

Many mammalian studies have established the relationship between insulin and its plasma-membrane-located receptor (Cuatrecasas, 1974). The hormone–receptor complex was shown to be directly linked to the cellular action of insulin by a mechanism which remains unclear (Walaas & Horn, 1981). In mammals, it was recognized that under conditions of obesity and maturity-onset-type diabetes, endogenous insulin showed reduced binding to its receptor (Olefsky, 1976; Wigand & Blackard, 1979) and this effect has been attributed to both reduced number and affinity of the plasma membrane receptors for endogenous insulin (Bar *et al.* 1976; Kahn, 1979). Thus, it was shown that down regulation of insulin receptors in mammals could be effected under conditions of hyperinsulinaemia (Blackard *et al.* 1978) and it appeared from both in vivo and in vitro evidence that the basal level of the hormone ultimately regulates the insulin receptor in a reciprocal relationship (Kahn, 1979). Among a variety of factors which affect this balance, it was established that reduced insulin binding could be induced either acutely, following glucose loading (Muggeo *et al.* 1977), or chronically, following administration of high-carbohydrate diets in several mammalian cell types including hepatocytes, monocytes erythrocytes and adipocytes (Freychet *et al.* 1972; Kahn *et al.* 1973; Kolterman *et al.* 1979; Robinson *et al.* 1979). The significance of this phenomenon to the present study centred on the possibility that in rainbow trout (*Salmo gairdneri*) the role of insulin was less directly oriented towards control of carbohydrate metabolism.

In previous studies, we have shown that although insulin did promote clearance of blood glucose towards oxidative pathways, no stimulation of hepatic or skeletal musculature glycogenesis was observed (Ablett *et al.* 1981*a*). Additionally, compared with radiolabelled

* Part of a dissertation submitted by R.F.A. to Oregon State University in partial fulfilment of the requirements for the PhD degree.

† Technical paper no. 6361, Oregon Agricultural Experiment Station, Oregon State University, Corvallis, Oregon 97331, USA.

Present addresses: ‡Department of Nutritional Sciences, Faulty of Medicine, University of Toronto, Ontario, Canada M55 1A8. ‖Environmental Safety Division, Proctor and Gamble Co., Cincinnatti, Ohio 45217, USA.

§ For reprints.

Table 1. *Composition (g/kg) and metabolizable energy (ME) of experimental diets fed to rainbow trout* (Salmo gairdneri)

Diet ... Ingredient	Control	High protein	High carbohydrate
Casein	495·0	620·0	310·0
Gelatin	87·0	80·0	90·0
Dextrin	156·0	0·0	300·0
Salmon oil	100·0	90·0	90·0
Duragel*	0·0	6·0	6·0
Alpha cellulose	82·0	70·0	55·0
Carboxymethyl cellulose	10·0	10·0	10·0
Choline chloride (700 g/kg)	10·0	10·0	10·0
Mineral mix†	40·0	40·0	40·0
Vitamin mix†	20·0	20·0	20·0
Amino acid supplement‡	0·0	0·0	15·0
Total ME‖ (kJ/g)	13·88	14·42	11·54
Protein (kJ/g)	9·48	11·41	6·52
Carbohydrate (kJ/g)	1·04	0·00	2·01
Fat (kJ/g)	3·35	3·01	3·01

* A. E. Staley, MFG Co., Decatur, IL.
† Sinnhuber *et al.* (1977).
‡ Contained (g/kg): arginine 6; cysteine 2; methionine 4; DL-tryptophan 3.
‖ In calculating ME, it was assumed that dextrin gives 6·7, protein 16·3 and fat 33·5 kJ ME/g (Phillips & Brockway, 1959).

glucose, insulin stimulated greater over-all retention of radiolabelled leucine in protein and lipid compartments (Ablett *et al.* 1981*b*). Thus, in other studies with teleosts, a general lack of blood glucose control, low storage level of glycogen, generally low carbohydrate utilization and predominant amino acid stimulation of insulin release also indicated a more significant role of the hormone in the control of other dietary metabolities, notably protein precursors (Black *et al.* 1960; Singh & Nose, 1967; Palmer & Ryman, 1972: Ince, 1980). On the basis of the mammalian evidence, one might expect less-pronounced depression of insulin binding in rainbow trout following chronic administration of high-carbohydrate diets and, by homology, to observe reduced insulin-receptor binding as a result of a chronically-administered high-protein diet.

Since previous studies in rainbow trout have examined the action of the hormone only at pharmacological dose levels (Cowey *et al.* 1977*a*, *b*; Ablett *et al.* 1981*a*, *b*) this present study provided a model for investigating insulin action at the physiological level in fish reared on either control, high-carbohydrate or high-protein diets. Further, because the peripheral skeletal musculature forms the major target organ of insulin, and no previous investigations of insulin-binding activity have been made in purified skeletal muscle plasma membranes, it was considered important to determine insulin-binding activity in this tissue. Additional comparative information was also provided by binding studies performed on isolated hepatocytes prepared from trout reared on the same experimental diets.

EXPERIMENTAL

Animals

Mount Shasta strain rainbow trout spawned at the Food Toxicology and Nutrition Laboratory, Oregon State University, with an initial weight of 30 g, were reared over a period of 15 months on three iso-energetic, semi-purified diets formulated to provide either

Table 2. *Effect of diets on the growth of rainbow trout* (Salmo gairdneri) *given high-protein and high-carbohydrate diets*

(Mean values and standard deviations for twelve fish at 15 months)

Diet* ...	Control		High protein		High carbohydrate	
	Mean	SD	Mean	SD	Mean	SD
Body-wt (g)	$382{\cdot}8^{a}$	104·0	$503{\cdot}8^{b}$	62·8	$194{\cdot}0^{b}$	56·2
Length (m)	$0{\cdot}302^{a}$	0·017	$0{\cdot}308^{a}$	$0{\cdot}010^{a}$	$0{\cdot}256^{b}$	0·017
Condition factor†	$1{\cdot}34^{a}$	0·23	$1{\cdot}71^{b}$	0·17	$1{\cdot}14^{a}$	0·29

* For details, see Table 1.

† Condition factor = body-weight × 100 ÷ length^{3}.

^{a,b} Values in horizontal rows with different superscripts were significantly different ($P < 0{\cdot}05$).

control, high-protein or high-carbohydrate rations (Table 1). High-protein diets contained no carbohydrate, but a maintenance quantity of protein (400 g/kg) was essential in the high-carbohydrate diet since rainbow trout do not survive in its absence. The control-diet was composed of intermediate protein (600 g/kg) and carbohydrate (150 g/kg).

All fish were maintained in 900 mm^{3} glass-fibre tanks receiving well-water (12°) at a flow rate of 7·6 l/min and fed *ab lib.* twice daily. Tissues were sampled between 9 and 15 months and at the end of this period weight and fork-length measurements were recorded for remaining stocks (Table 2).

Tissue preparations

Skeletal muscle plasma membranes. Pooled samples of skeletal muscle plasma membranes, isolated and characterized as previously described (Ablett, 1982), were prepared from portions of the caudal flank musculature of 100–300 g rainbow trout from each diet (Table 1). Protein content was evaluated by the method of Lowry *et al.* (1951) with bovine serum albumin (BSA) as the standard. Samples were stored at −40° before use in insulin-binding studies.

Isolated hepatocytes. Isolated hepatocytes were prepared from 100–300 g rainbow trout from each diet according to the method of Hazel & Prosser (1979). Cells were assessed for initial viability in a haemocytometer after dilution into Trypan blue (5 g/l incubation buffer). Lactate dehydrogenase (*EC* 1.1.1.27) leakage, determined by the method of Holbrook *et al.* (1975), was also used initially and at the end of binding experiments to evaluate the viability of cells. After assessment of protein (Lowry *et al.* 1951), all preparations were immediately used for insulin-binding studies.

Binding studies

Binding experiments with isolated skeletal muscle plasma membranes and hepatocytes were performed directly in 1·5 ml microcentrifuge tubes (West Coast Scientific, Berkeley, CA) in a total volume of 0·5 ml containing 0·1 ng [^{125}I]iodoinsulin (specific activity > 50 μCi/μg; Amersham Radiochemical Corp., Arlington Heights, IL)/ml. Unlabelled bovine insulin (Sigma Chemical Co., St Louis, MO) was added in 0·05 ml portions over a concentration range of 0–50 μg/ml. In all instances, binding studies were initiated by addition of either plasma membranes or hepatocytes and, after incubation to steady-state, studies were terminated by centrifugation of the reaction vials for 10 min at 10000 ***g*** in a Sorvall Superspeed RC2-B centrifuge. Supernatant fractions containing unbound insulin were

transferred by pipette to 4·5 ml Aquasol (New England Nuclear, Boston, MA) and radioactivity measured using a Beckman LS 7500 scintillation counter adjusted for ^{125}I detection. Similarly, radioactivity in precipitates containing the bound fraction was measured following overnight digestion in 0·3 ml NCS (Amersham Radiochemical Corp.) with subsequent transfer to 4·5 ml toluene scintillation fluor containing 6·0 g PPO and 0·05 g POPOP/l.

To determine optimal pH requirements, preliminary insulin-binding studies in the presence of 1 ng/ml or 50 μg/ml bovine insulin were conducted on portions containing 75–100 μg protein of initial-filtered homogenates of skeletal muscle and isolated hepatocytes from control-diet-reared trout. Skeletal muscle filtrate assays were conducted in 0·5 mM-Tris-hydrochloride, 30 g BSA/l. Isolated hepatocyte assays were conducted in 100 mM-sodium chloride, 40 mM-HEPES, 20 mM-potassium chloride, 10 mM-glucose, 8 mM-sodium bicarbonate, 2·5 mM-calcium chloride, 1 mM-magnesium sulphate, 20 g BSA/l. Appropriate pH adjustments were made immediately prior to assay in all instances. In the same way and with the same amounts of protein and buffer composition, temperature requirements to attain steady-state binding were established at 4, 12, 23 and 37° for skeletal muscle filtrate and isolated hepatocyte preparations in the presence of bovine insulin at 1 ng/ml and 50 μg/ml. Care was taken to ensure that pH 7·4 was maintained in all assays over the temperature ranges used.

Insulin-binding experiments with skeletal muscle plasma membranes were performed on 100–125 μg protein samples, using 0·5 mM-Tris-hydrochloride; 30 g BSA/l, pH 7·4 as an incubation medium. Hepatocyte-binding studies were performed on 20–30 μg protein in an incubation medium composed of 100 mM-sodium chloride, 40 mM-HEPES, 20 mM-potassium chloride, 10 mM-glucose, 8 mM-sodium bicarbonate, 2·5 mM-calcium chloride, 1 mM-magnesium sulphate, 20 g BSA/l, pH 7·4. In all instances, non-specific binding, defined as the radioactivity associated with the pellet in the presence of a large excess of unlabelled bovine insulin (50 μg/ml) was subtracted from total [^{125}I]iodoinsulin binding to yield specific-binding values.

Statistics

Growth differences attributed to dietary regimens were evaluated against the control diet by Student's *t* test. Differences in mean values were considered significant with $P < 0·05$. No comparisons were made between tissue preparations or between high-protein and high-carbohydrate diets.

RESULTS

Growth measurements

As shown in Table 2, after 15 months high-protein-reared trout produced a 31·6% higher body-weight and an elevated condition factor compared with control diets. Under the same conditions, body-weights of high-carbohydrate-reared trout were 49·3% less than that of controls.

Viability of isolated hepatocytes

In all studies performed, initial viability of isolated hepatocytes averaged 85·6 (SD 9·0)% by Trypan blue exclusion. Viability determinations by lactate dehydrogenase leakage initially averaged 91·6 (SD 2·3)% and this declined to 86·4 (SD 2·8)% after 3 h, representing an acceptable 5·6% reduction of viability through the experimental period.

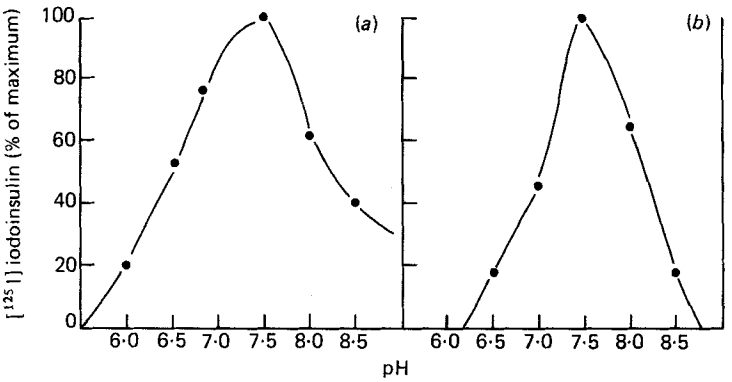

Fig. 1. Effect of pH on specific [^{125}I]iodoinsulin in binding (% of maximum) in (*a*) skeletal muscle filtrate and (*b*) isolated hepatocytes from rainbow trout (*Salmo gairdneri*) reared on the control diet (see Table 1). All values represent the mean of triplicate assays performed in the presence of 1 ng/ml bovine insulin. Non-specific binding values obtained in the presence of 50 μg/ml bovine insulin were subtracted and in no instance exceeded 3·6%. Maximal specific binding (mean with SD) was 8·2 (0·6)% for skeletal muscle filtrate and 11·5 (1·0)% for isolated hepatocytes.

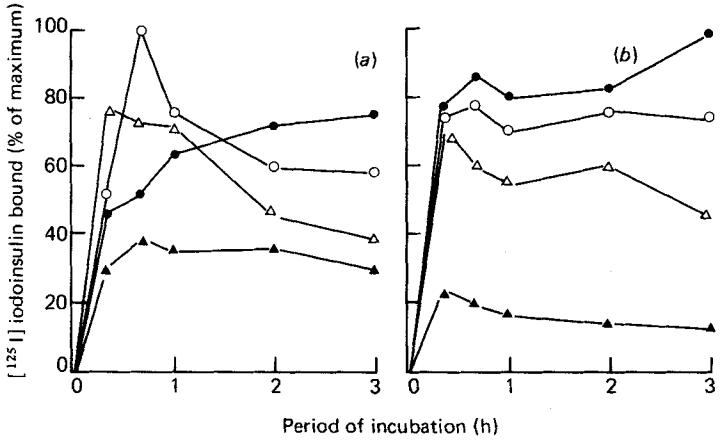

Fig. 2. Effect of temperature on specific [^{125}I]iodoinsulin binding (% of maximum) in (*a*) skeletal muscle filtrate and (*b*) isolated hepatocytes from rainbow trout (*Salmo gairdneri*) reared on the control diet (see Table 1). Incubation at the time intervals specified at: (●), 4°; (○), 12°; (△), 23°; (▲), 37°. All values represent the mean of triplicate assays performed in the presence of 1 ng/ml bovine insulin. Non-specific binding values obtained in the presence of 50 μg/ml bovine insulin were subtracted and in no instance exceeded 3·0%. Maximal specific binding (mean with SD) was 8·8 (0·9)% for skeletal muscle filtrate and 11·2 (1·2)% for isolated hepatocytes.

Insulin-binding studies

pH dependence. The binding of [^{125}I]iodoinsulin to skeletal muscle filtrate and isolated hepatocytes was maximal at pH 7·5 and showed a sharp decline on either side of this value (Fig. 1 *a*, *b*). Compared with isolated hepatocytes, slightly greater [^{125}I]iodoinsulin binding was observed in the skeletal muscle filtrate on the acidic side of the pH curve.

Temperature dependence. [^{125}I]iodoinsulin binding to the filtrate fraction of skeletal muscle and also isolated hepatocytes was profoundly affected by temperature and in both instances greatest specific binding was seen at 4° after 3 h (Fig. 2). For skeletal muscle filtrate, binding was achieved more rapidly at higher temperatures and showed a greater decline with

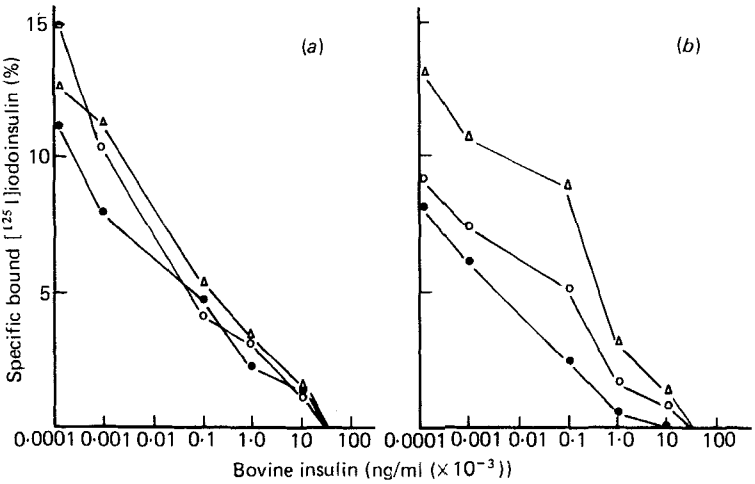

Fig. 3. Specific [^{125}I]iodoinsulin binding (%) to (*a*) skeletal muscle plasma membranes and (*b*) isolated hepatocytes of rainbow trout (*Salmo gairdneri*) reared on control, (○), high-protein (●), and high-carbohydrate (△) diets (see Table 1). All values represent the mean of three assays performed in triplicate in the presence and absence of unlabelled bovine insulin (0–50 μg/ml). Non-specific binding obtained in the presence of 50 μg/ml bovine insulin was subtracted and in no instance exceeded 4·2%. All values from high-carbohydrate diet hepatocytes were significantly different from those for control-diet hetaptocytes by Student's *t* test ($P < 0{\cdot}05$).

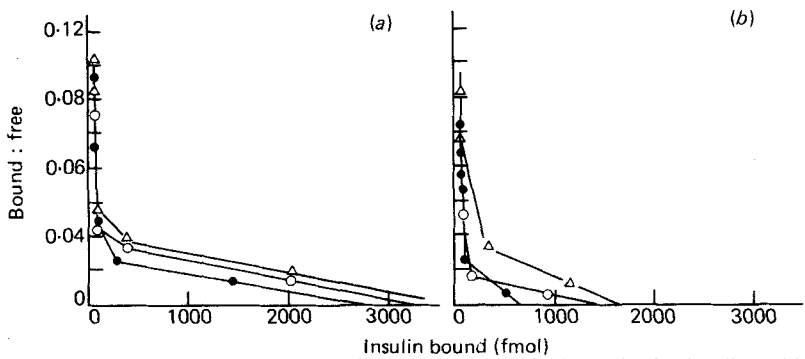

Fig. 4. Scatchard plot analysis of specific-binding values shows binding of bovine insulin to (*a*) skeletal muscle plasma membranes and (*b*) isolated hepatocytes of rainbow trout (*Salmo gairdneri*) reared on control (○), high-protein (●) and high-carbohydrate (△) diets (see Table 1).

prolonged incubation, such that the steady-state ultimately achieved was inversely proportional to the temperature (Fig. 2*a*). Isolated hepatocytes yielded similar results, except that the rates of binding at 4 and 12° were initially more rapid and for the latter showed less decline with time compared with skeletal muscle preparations (Fig. 2*b*).

Effects of diet on insulin binding. As indicated in Fig. 3, comparable levels of specific [^{125}I]iodoinsulin binding were observed between tissue types with a maximum level of 15% observed in skeletal muscle plasma membranes. In all instances, increasing concentrations of bovine insulin competitively displaced [^{125}I]iodoinsulin in an apparently sigmoidal relationship which was indicative of negative cooperativity, that is, the affinity of the receptor decreased with increasing occupancy. This phenomenon has been well established as a binding activity of the insulin to the receptor (De Meyts *et al.* 1976).

No differences in [^{125}I]iodoinsulin binding were apparent between dietary regimens for

Table 3. *Apparent affinity constants for skeletal muscle plasma membranes and isolated hepatocytes of rainbow trout* (Salmo gairdneri)

(Mean values and standard deviations from three experiments. Values derived from Fig. 4)

Diet† ...	Control		High protein		High carbohydrate	
	Mean	SD	Mean	SD	Mean	SD
Skeletal muscle						
High affinity (mol ($\times 10^{-10}$))	0·60	0·15	0·98	0·27	0·52	0·08
Low affinity (mol ($\times 10^{-9}$))	1·20	0·21	0·97	0·18	1·25	0·28
Hepatocytes						
High affinity (mol ($\times 10^{-10}$))	3·45	1·13	5·90	2·54	2·98	0·31
Low affinity (mol ($\times 10^{-9}$))	2·14	0·70	3·82	1·92	2·66	0·45

Values did not differ significantly from control values by Student's t test ($P > 0{\cdot}05$).
† For details, see Table 1.

Table 4. *Insulin receptor concentrations (sites ($\times 10^{-11}$)/mg protein) for skeletal muscle plasma membranes and isolated hepatocytes of rainbow trout* (Salmo gairdneri)

(Mean values and standard deviations from three experiments. Values derived from Fig. 4)

Diet† ...	Control		High protein		High carbohydrate	
Source of receptors	Mean	SD	Mean	SD	Mean	SD
Skeletal muscle plasma membrane	176[a]	18	160[a]	21	200[a]	12
Isolated hepatocytes	240[a]	44	160[a]	34	399[b]	53

[a, b] Values across rows with different superscripts are significantly different from the control values ($P < 0{\cdot}05$).
† For details, see Table 1.

the skeletal muscle plasma membranes, except for an apparently lowered binding of [^{125}I]iodoinsulin at low bovine insulin concentrations (0·1–100 ng/ml) in the trout reared on the high-protein diet (Fig. 3*a*). This trend, which did not differ significantly from that of the control trout ($P > 0{\cdot}05$), was also observed in isolated hepatocytes ($P > 0{\cdot}05$). A marked elevation of specific binding observed in isolated hepatocytes of high-carbohydrate-reared fish ($P < 0{\cdot}05$) indicated that this treatment bound more insulin compared with isolated hepatocytes from trout reared on control diet (Fig. 3*b*).

Scatchard plot analysis of the 'binding' values revealed curvilinear binding responses for all preparations and this provided further evidence of negative co-operativity (Fig. 4). With the assumption of a two-site model for insulin binding (Cech *et al.* 1980), apparent high- and low-affinity constants were calculated by linear regression of the upper and lower portions of the Scatchard analysis (Fig. 4). Values of high and low affinity for skeletal muscle plasma membranes and isolated hepatocytes differed by a factor of ten but, compared with controls, no significant differences attributed to diets were observed at either level (Table 3).

Insulin-receptor concentrations (Table 4) were calculated from intercepts on the abscissa of the Scatchard analyses (Fig. 4) and, except for the isolated hepatocytes of high-carbohydrate-reared trout, these results (Fig. 4*b* and Table 4) showed no differences between dietary treatments. Hepatocytes of high-carbohydrate-reared trout showed a significantly increased receptor concentration compared with control diets ($P < 0{\cdot}05$). This indicated that the enhanced binding of insulin in the high-carbohydrate hepatocytes (Fig. 3*b*) was mainly due to an increased number of binding sites and not to an altered affinity for insulin.

DISCUSSION

Specificity of insulin binding

In the present study, results were obtained that were generally consistent with our hypothesis that dietary effects on insulin binding in rainbow trout might vary from that established for mammals.

Maximal steady-state insulin binding with a temperature dependence of 4° at 3 h (Fig. 2) and pH optimum of 7·5 (Fig. 1) in skeletal muscle filtrate and isolated hepatocytes agreed with erythrocyte studies previously conducted in Atlantic hagfish (*Myxine glutinosa*) and brown trout (*Salmo trutta*) (Muggeo *et al.* 1979*a*, *b*). In the latter species, values comparable to those for insulin binding of erythrocytes at both 4 and 22° for trout were observed over a 4 h incubation period at pH 7·4. The use of low temperatures (4°) for mammalian insulin-binding studies, notably in skeletal plasma membranes, was shown to minimize insulin-receptor degradation and also hydrolysis of insulin itself by cleavage enzymes present in tissue preparations (Yokono *et al.* 1979). Investigation of these events was beyond the scope of the present study, but it was felt that the integrity of insulin binding over a 3 h period was maintained by the low-temperature-dependent nature of rainbow trout preparations. A slight shift of the pH curve toward acidic values observed for skeletal muscle filtrate (Fig. 1*a*) may have reflected a pronounced dependence of rainbow trout skeletal musculature for glycolytic pathways (Fukuda, 1958). As such, one would expect minimal in vivo insulin binding to occur during activity and thus some tolerance of accumulative lactate would be physiologically advantageous to myotome-located insulin receptors.

Total specific insulin-binding recorded in isolated hepatocytes particularly and that of skeletal muscle plasma membranes, were of the same magnitude as those observed in mammalian (Terris & Steiner, 1975) and non-mammalian studies (Kemmler *et al.* 1978). Negative co-operativity in insulin receptors, indicated by the curvilinearity of Scatchard plots (Fig. 4), has been attributed either to one class of receptors yielding site–site interactions or to the presence of two or more classes of receptors with high- and low-affinity characteristics (Almira & Reddy, 1979). Expression of the receptor affinity for bovine insulin (Table 3) was based on the latter model and the apparent affinity constants recorded for isolated hepatocytes were of the same magnitude as observed in other species (Kemmler *et al.* 1978; Caro & Armatruda, 1980). In the present study, the slightly-raised high and low affinity of isolated hepatocytes for insulin in comparison with skeletal muscle (Table 3) might have reflected the important and central role of the liver in facilitated accommodation of metabolites by insulin for subsequent direction into storage or metabolic pathways.

Evolutionary changes in the insulin molecule have been observed (Steiner, 1976) and the presumption that corresponding receptor changes would be needed to maintain adequate hormonal recognition was logical. However, studies in a range of mammalian and non-mammalian species have excluded the possibility of species-specificity among insulin receptors (Kemmler *et al.* 1978). Thus, in brown trout, a variety of exogenous insulin types (bonito, porcine proinsulin, guinea-pig, chicken and bovine) were shown to bind with similar absolute affinities in the same negatively co-operative relationship (Muggeo *et al.* 1979*a*). The latter authors concluded that the receptor molecule was more functionally conserved than the hormone through phylogeny. These findings provided good evidence that bovine insulin was a suitable exogenous source of insulin for physiological binding studies in the absence of extracted rainbow trout insulin.

Receptor concentrations of isolated hepatocytes and skeletal muscle plasma membranes (Table 4) also compared favourably with values obtained for liver plasma membranes of chicken, rat, mouse and guinea-pig in which values of 2·5, 25, 60 and 100 sites ($\times 10^{-11}$)/mg protein respectively were recorded (Kahn *et al.* 1974; Soll *et al.* 1975; Muggeo *et al.* 1979*a*).

Recent evidence has demonstrated the suitability of isolated hepatocytes for insulin-binding studies (Terris & Steiner, 1975; Caro & Armatruda, 1980) and the high viability of isolated hepatocytes recorded throughout binding experiments in the present study attested to the usefulness of this preparation for this type of study. Unfortunately, in the absence of comparative studies the validity of the values obtained for skeletal muscle plasma membranes must remain speculative at this time.

Effects of diet on insulin binding

Chronic administration of the high-carbohydrate diet caused an increased receptor concentration in isolated hepatocytes. This was accompanied in the high-protein diet by some depression of specific binding and receptor concentration, although the differences were not significant (Table 4). Several explanations might account for these findings. First, following the reciprocal relationship established between endogenous insulin levels and the insulin-receptor concentration for mammals (Kahn, 1979), the increased receptor concentration of high-carbohydrate treatments may have represented a normalization response to lowered circulatory insulin levels invoked by the reduced protein level (400 g/kg) in the high-carbohydrate diet of similar energy content. Accumulating evidence in teleosts shows that amino acids (in preference to glucose) constitute the major stimulus for insulin release from islet tissue (Ince & Thorpe, 1977; Ince, 1979, 1980). Therefore, one might have expected to observe chronically-reduced circulatory-insulin levels with the high-carbohydrate treatments. In rainbow trout given high-carbohydrate diets for 6 weeks, Cowey *et al.* (1977*b*) observed that raised plasma glucose levels attributed to the diet were sensitive to administration of exogenous bovine insulin. This suggested that ambient endogenous insulin levels were low. Further, these authors noted a markedly lower plasma glucose level in rainbow trout given high-protein diets for 6 weeks that did not decrease following administration of exogenous bovine insulin. It was inferred that elevated endogenous insulin due to high levels of circulatory amino acids derived from the high-protein diet was responsible for elevated endogenous insulin levels. Thus, the endogenous insulin maintained a lowered plasma glucose level and coincidentally negated the effects of the exogenous bovine insulin. In support of this, a study of rainbow trout has demonstrated an elevation of plasma insulin levels following administration of a high-protein diet for 3 weeks (Ahmad & Matty, 1975). In the present study, we suggest that the possibility of chronically-elevated endogenous insulin promoted by a high-protein diet may have led to reduced receptor binding in both skeletal muscle and isolated hepatocytes of high-protein-reared fish, although the effects observed were not significant. It was not surprising that the differences in insulin binding between control and high-protein (700 g/kg)-reared fish were not great since the control diets routinely used in our laboratory contain 600 g protein/kg (Table 1).

As an alternative explanation for the results, we postulate that the increased receptor concentration of the liver tissue in high-carbohydrate treatments might be associated with the poor growth rate observed in this group (Table 2). No doubt this latter effect was due to a lowered metabolizable energy availability (Table 1) of the iso-energetic diet attributable to a combined low level of protein and a low efficiency for carbohydrate utilization (Nagai & Ikeda, 1971; Austreng *et al.* 1977). Among poikilotherms it was clearly demonstrated that over-all energy demand was greater for smaller individuals of the same species (Zeuthen, 1953). Thus, in a state of deprived nutrition, optimization of metabolic energy production by facilitating either cellular glucose uptake or amino acid uptake and energy synthesis by gluconeogenic pathways, would be a plausible strategy to meet these requirements (Renaud & Moon, 1980). In this instance, unlike the situation for mammals, the use of glucose as a substrate was considered less likely. Cowey *et al.* (1977*b*) have

previously shown that the hepatic phosphorylating capacity in rainbow trout by glucokinase (*EC* 2.7.1.2) and hexokinase (*EC* 2.7.1.1) was not increased after chronic adminstration of high-carbohydrate diets. Thus gluconeogenesis might be favoured, even with a low availability of circulatory amino acids. In rainbow trout, this pathway was shown to occur almost entirely in the liver and to a lesser extent in kidney tissue (Walton & Cowey, 1979*a*, *b*). This would clearly explain why increased insulin-receptor concentration of the liver in high-carbohydrate treatments was not accompanied by a raised concentration in the glycolytically-directed skeletal muscle tissue. Moreover, under conditions of plentiful amino acid availability, as seen in control and high-protein diets, this permissive role of receptor concentration in enhancing hepatic amino acid uptake would be less vital and thus lowered receptor concentrations might be observed in a reciprocal relationship with the amino acid supply. This effect was apparent in the present study (Fig. 3).

The results obtained did provide evidence apparently at deviance from the established mammalian pattern of insulin-receptor binding under the influence of diet. This preliminary study provides the basis for further investigation of this interesting and phylogenetically-important aspect of insulin action.

The technical assistance of Messrs John Casteel, Theodore Will and David Nowac in caring for fish stocks throughout the duration of this experiment is greatly appreciated, as is the valuable laboratory assistance of Janet Williams and Janet Wilcox. This work was funded by a grant from the American Diabetes Association, Oregon Affiliate.

REFERENCES

Ablett, R. F. (1982). An investigation of the effects of nutritional status on the action of insulin in rainbow trout (*Salmo gairdneri*). PhD Thesis, Oregon State University.

Ablett, R. F., Sinnhuber, R. O., Holmes, R. M. & Selivonchick, D. P. (1981*a*). *General and Comparative Endocrinology* **43**, 211–217.

Ablett, R. F., Sinnhuber, R. O., & Selivonchick, D. P. (1981*b*). *General and Comparative Endocrinology* **44**, 418–427.

Ahmad, M. M. & Matty, A. J. (1975). *Pakistan Journal of Zoology* **7**, 1–6.

Almira, E. C. & Reddy, W. J. (1979). *Endocrinology* **104**, 205–211.

Austreng, E., Risa, S., Edwards, D. J. & Hvidsten, H. (1977). *Aquaculture* **11**, 39–50.

Bar, R. S., Gorden, P., Roth, J., Kahn, C. R. & De Meyts, P. (1976). *Journal of Clinical Investigation* **58**, 1123–1128.

Black, E. C., Robertson, A. C., Hinslip, A. R. & Chiu, W. G. (1960). *Journal of the Fishery Research Board of Canada* **17**, 487–499.

Blackard, W. G., Guzelian, P. S. & Snell, E. E. (1978). *Journal of the Clinical Climatology Association* **90**, 94–101.

Caro, J. F. & Armatruda, J. M. (1980). *Journal of Biological Chemistry* **255**, 10052–10055.

Cech, M. J., Freeman, R. B., Caro, J. F. & Armatruda, J. M. (1980). *Biochemical Journal* **188**, 839–843.

Cowey, C. B., De La Higuera, M. & Adron, J. (1977*a*). *British Journal of Nutrition*, **38**, 385–395.

Cowey, C. B., Knox, D., Walton, M. J. & Adron, J. (1977*b*). *British Journal of Nutrition* **38**, 463–470.

Cuatrecasas, P. (1974). *Annual Review of Biochemistry* **43**, 169–214.

De Meyts, P., Bianco, A. R. & Roth, J. (1976). *Journal of Biological Chemistry* **25**, 1877–1888.

Freychet, P., Laudat, M. H., Laudat, P., Rosselin, A., Kahn, C. R., Gorden, P. & Roth, J. (1972). *Federation of European Biochemical Societies, Letters* **25**, 339–342.

Fukuda, H. (1958). *Bulletin of the Japanese Society of Scientific Fisheries* **24**, 24–32.

Hazel, J. R., & Prosser, C. L. (1979). *Journal of Comparative Physiology* **134**, 321–329.

Holbrook, J. J., Liljas, A., Steindel, S. J. & Rossman, M. G. (1975). In *The Enzymes*, p. 191 [P. D. Boyer, editor]. New York: Academic Press.

Ince, B. W. (1979). *General and Comparative Endocrinology* **37**, 533–540.

Ince, B. W. (1980). *General and Comparative Endocrinology* **40**, 275–282.

Ince, B. E. and Thorpe, A. (1977). *General and Comparative Endocrinology* **31**, 249–256.

Kahn, C. R. (1979). *Proceedings of the Society for Experimental Biology and Medicine* **162**, 13–21.

Kahn, C. R., Freychet, P., Neville, D. M. & Roth, J. (1974). *Journal of Biological Chemistry* **249**, 2249–2257.

Kahn, C. R., Neville, D. M. & Roth, J. (1973). *Journal of Biological Chemistry* **248**, 244–250.

Kemmler, W., Renner, R., Zynamon, A. & Hepp, K. D. (1978). *Biochimica et Biophysica Acta* **543**, 349–356.

Kolterman, O. G., Greenfield, M., Reaven, G. M. Saekow, M. & Olefsky, J. M. (1979). *Diabetes* **28**, 731–736.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *Journal of Biological Chemistry* **193**, 265–275.

Muggeo, M., Bar, R. S. & Roth, J. (1977). *Journal of Clinical Endocrinology and Metabolism* **44**, 1206–1209.

Muggeo, M., Ginsberg, B. H., Roth, J., Neville, D. M., De Meyts, P. & Kahn, C. R. (1979*a*). *Journal of Endocrinology* **104**, 1393–1402.
Muggeo, M., Van Obberghen, E., Kahn, C. R., Roth, J., Ginsberg, B. H., De Meyts, P., Emdin, S. O., & Falkmer, S. (1979*b*). *Diabetes* **28**, 175–181.
Nagai, M. & Ikeda, S. (1971). *Bulletin of the Japanese Society of Scientific Fisheries* **37**, 404–414.
Olefsky, J. M. (1976). *Diabetes* **25**, 1154–1165.
Palmer, T. N. & Ryman, B. E. (1972). *Journal of Fish Biology* **4**, 311–319.
Phillips, A. M. & Brockway, D. R. (1959). *Progressive Fish Culture* **21**, 3–16.
Renaud, J. M. & Moon, T. W. (1980). *Journal of Comparative Physiology* **135**, 127–137.
Robinson, T. J., Archer, J. A., Gambhir, K. K., Hollis, V. W., Carter, L. & Bradley, C. (1979). *Science* **205**, 200–202.
Singh, R. P. & Nose, T. (1967). *The Bulletin of the Freshwater Fisheries Research Laboratory (Tokyo)* **17**, 21–25.
Sinnhuber, R. O., Wales, J. H., Hendricks, J. D., Putnam, G. B., Nixon, J. E. & Pawlowski, N. E. (1977). *Mycotoxins in Human and Animal Health*, p. 743 Park Forest, IL: Pathotox Publishers.
Soll, A. H., Kahn, C. R. & Neville, D. M. (1975). *Journal of Biological Chemistry* **250**, 4702–4708.
Steiner, D. (1976). *Diabetes* **26**, 322–337.
Terris, S. & Steiner, D. F. (1975). *Journal of Biological Chemistry* **250**, 8389–8398.
Walaas, O. & Horn, R. S. (1981). *Trends in Pharmacological Sciences* **2**, 196–198.
Walton, M. J., & Cowey, C. B. (1979*a*). *Comparative Biochemistry and Physiology* **62B**, 75–79.
Walton, M. J., & Cowey, C. B. (1979*b*). *Comparative Biochemistry and Physiology* **62B**, 497–500.
Wigand, J. P. & Blackard, W. G. (1979). *Diabetes* **28**, 287–291.
Yokono, K., Imamura, Y., Sakai, H. & Baba, S. (1979). *Diabetes* **28**, 810–817.
Zeuthen, E. (1953). *Quarterly Review of Biology* **28**, 1–12.

Printed in Great Britain