Comparative Biochemistry and Physiology, Part B 199 (2016) 43–49

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part B

journal homepage: www.elsevier.com/locate/cbpb

How Tom Moon's research highlighted the question of glucose tolerance in carnivorous fish☆

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ARTICLE INFO

Article history:
Received 18 June 2015
Received in revised form 30 October 2015
Accepted 1 November 2015
Available online 3 November 2015

Keywords:
Fish
Glucose metabolism
Nutrients
Antidiabetic drugs
Hepatocytes

ABSTRACT

Fifteen years ago, Tom Moon wrote a review on this journal in order to propose some explanations to the exacerbated glycaemic response after a glucose load or a carbohydrate meal intake observed in fish, the so-called intolerance to glucose. Before, but in most of cases after this paper, several laboratories worldwide started to make important efforts in order to better understand this strange phenotype observed in fish and that so far seemed to belong to diabetic humans only. Tom had been worked on fish metabolism for at least 30 years when he proposed that mini-review and the paths opened by him in 2001 were followed by tens of fish researchers, making this paper a breaking point on the field. Fifteen years later, we propose not only to have a look to the answers given to the questions rose in that paper, but also to summarize how his career over all these years impacted the domain of glucose metabolism in fish. In the review, we will show how Tom Moon analysed at different levels (from genes up to the whole organism), using distinct experimental tools (cells, hormone or glucose injection, pumps, drugs) the questions of glucose metabolism, tolerance and nutrition in fish species.

1. Introduction

In 2001, Tom Moon wrote a review facing one of the most intriguing questions interesting scientist that worked on fish metabolism by then: the intolerance to glucose (Moon, 2001). It seemed that following a glucose load or a carbohydrate-enriched meal, carnivorous fish (like salmonids and other) displayed a long and slow glucose clearance, with high blood glucose levels that could last up to 24 h (Harmon et al., 1991; Mazur et al., 1992; Wright et al., 1998). Obviously, this response seemed disproportioned when compared to mammals, but they could not explained by the temperature differences only (Polakof et al., 2012). These observations led several laboratories worldwide to made tremendous efforts to understand the metabolic mechanisms behind this behaviour and to apply this knowledge to the development of new fish diets for aquaculture including more carbohydrates and less protein, which imply major economic and environmental implications (Wilson, 1994; Hemre et al., 2002; Krogdahl et al., 2005; Panserat et al., 2013).

Tom had been worked on fish metabolism for 30 years when he proposed that mini-review and face the glucose intolerance question by putting some new ideas on the table. The paths opened by him in 2001 were followed by tens of fish researchers (cumulating more than 190 citations up today) and his review marked a breaking point on the field. Fifteen years later, we propose to have a look to the answers given to the questions rose in that paper, but also to summarize how his career over all these years impacted the field of glucose metabolism in fish. In the following chapters, we will show how Tom Moon analysed at different levels (from genes up to the whole organism) and using distinct experimental tools (cells, hormone or glucose injection, pumps, drugs) the questions of glucose metabolism, tolerance and nutrition in fish.

1.1. New approaches developed by Tom Moon's studies to explore the glucose metabolism in fish

1.1.1. The utilisation of freshly isolated hepatocytes as a tool for understanding glucose metabolism regulation in fish

The utilisation of *in vitro* models to explore fish metabolism has become an essential approach to unravel *in vivo* hidden mechanisms and by-pass the whole body hormone and factors regulation. Tom has devoted an important part of his career to develop with several colleagues a model of freshly isolated hepatocytes for studying fish metabolism (Moon et al., 1985; Mommsen et al., 1994; Moon, 2004). Today, this model is exploited by some of the most important fish laboratories worldwide. In this review, we will just focus on the utilisation of such a model for the exploration of glucose metabolism in particular. Some of the first studies started in the very early eighties, with the setup of the

☆ Contribution to a special issue celebrating the work of Dr. Thomas W. Moon on the occasion of his retirement after 45 years in comparative biochemistry and physiology.

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http://dx.doi.org/10.1016/j.cbpb.2015.11.001
1096-4959/

American eel (*Anguilla rostrata* LeSueur) hepatocyte isolation (Renaud and Moon, 1980a,b). For this particular species, these studies showed for the first time in fish that the hepatocyte gluconeogenesis was stimulated by lactate, glycerol and alanine. Further, this effect was potentiated in the presence of glucagon and allowed to spare the glycogen stores. Later studies on hepatocytes from other fish species further supported the robustness of the model, showing that as in *in vivo* trials, the fish hepatocyte respond to food deprivation by increasing glucose production and gluconeogenesis (Foster and Moon, 1993a).

May be the main advantage of the fish hepatocytes model developed by Tom and colleagues was the fact that they could be used as a tiny laboratory to explore hormone impact on fish glucose metabolism. The hepatocytes from sea raven (*Hemitripterus americanus*) were used as some of the first models for exploring *in vitro* the insulin/glucagon antagonism in fish. In 1987, he showed with Glen Foster (Foster and Moon, 1987) that the relationship between these two hormones was not fully comparable to that observed in mammals, most likely due to the carnivorous nature of the fish species approached. Nevertheless, some of the dogmatic mammalian effects were observed in fish as well, like the ability of glucagon to increase glucose production from glycogen and gluconeogenic precursors, and the positive action of insulin on glycogen synthesis from amino acids. Among the less classical hormones, also the GLP-1 was explored, showing a marked capacity to increase glucose production from both glycogenolysis and gluconeogenesis in several fish species (Mommsen and Moon, 1990). Surprisingly, the response was often even more potent than that observed with glucagon, supporting the idea that GLP-1 would be a better glucagon than glucagon itself (Moon, 2004).

On the same line, the utilisation of fish hepatocytes also allowed to carry out studies on the metabolic capacities of these extracted cells, especially at the enzyme level. Very valuable studies were performed by Tom and colleagues in order to better understand the regulation of enzymes participating at the glycolysis, gluconeogenesis and glycogen metabolism. Some of these studies provided the first evidence that hepatocytes from carnivorous fish do not necessarily followed the mammalian pattern from a glucose metabolism point of view (Mommsen et al., 1991). They showed that the rainbow trout (*Oncorhynchus mykiss*) and haghfish (*Myxine glutinosa*) (Foster et al., 1993b) liver had a quite heterogeneous cellular pattern concerning the enzymes of the carbohydrate metabolism, including a dominating gluconeogenic and oxidative metabolism in some hepatocytes, and a preference for glycogen breakdown and glycolysis in other. In 1990, he showed that in *H. americanus* most of the enzymes related to glucose metabolism were targets of insulin and glucagon (Foster and Moon, 1990a): while glucagon seemed to impact several glycolytic (PK, 6PF1K, LDH) and gluconeogenic (PEPCK) targets, the insulin action was reduced to the regulation of 6PF1K activity. This poor insulin action on carbohydrate enzymes was further explored in other species, like rainbow trout, at the molecular level *in vivo* (Polakof et al., 2010b) and *in vitro* (Lansard et al., 2010). However, the results were not always consistent with those of *H. americanus*: therefore, while insulin was unable to regulated 6PF1K gene expression, the PEPCK mRNA levels were strongly reduced in the presence of this hormone. Although these results are consistent with those reported in mammals, they represent a molecular potential only, and therefore should be confirmed at the enzyme activity level.

Finally, the hepatocyte system was intensively used by Tom to investigate particular mechanisms concerning the regulation of glycogen metabolism. Early studies on eel hepatocytes (*A. rostrata*) reported a strong and systematic glycogen depletion in isolated fish hepatocytes (Foster and Moon, 1990b). Later observations revealed that this effect could be accelerated by glucagon and prevented by insulin. The mechanisms was partially unravelled a few years later, when they discovered that insulin was able to reduce the activity of GPase (responsible of glycogen breakdown) and that this effect was dependent on the glucose present in the medium (Pereira et al., 1995b). Further, they also showed that this enzyme was regulated at the phosphorylation level and demonstrated that the study of such regulation status would be a more pertinent marker of glycogenolysis than the measurement of the end-products of the reaction (Mommsen et al., 1988; Moon et al., 1999).

1.1.2. Delivering hormones and drugs with osmotic pumps: does the chronic exposition matter?

One of the main concerns submitted by Tom on 2001 was the role of insulin in the glucose intolerance experienced by carnivorous fish after a glucose load or a rich-carbohydrate meal. In order to shed some light on this dark issue, the complex interaction between insulin action in the context of a high carbohydrate intake was explored by combining a nutritional trial with a pharmacological approach (Polakof et al., 2010a). The hypothesis pointed to a weak insulin sensitivity in trout in response to a carbohydrate-rich meal when compared to a mammalian species. We therefore delivered bovine insulin (known to be efficient in fish) by using osmotic pumps, able to release physiological doses of the hormone (estimated at ~6 ng/mL) over a relatively long-term period. Two kind of protocols were developed to answer this question: first, a short-term insulin action on a single test meal (30 h of insulin infusion); and second, a longer period of insulin delivery (5 days) under a high carbohydrate feeding. While a lower postprandial glycaemia was indeed observed in the short-term insulin-infused fish when compared to those receiving only a saline solution, some of the short-term results were unexpected. Only minor changes were observed in the most expected insulin sensitive tissues (skeletal muscle and adipose tissue), like an increased expression of Glut4 in the muscle despite an increased phosphorylation of Akt. Unlike the muscle and the adipose tissue, the liver appeared as a major actor during the postprandial period at least in response to the exogenous insulin infusion. We did observe several metabolic changes that could help to explain the reduced blood circulating levels. Among them, the increased potential of several metabolic pathways known to drain glucose from the blood stream, such as the glycogenesis (+50% of glycogen in the insulin-infused fish) or *de novo* lipogenesis (up-regulation of FAS and the transcription factor associated, SREBP-1c). Further, other elements (like reduced mRNA levels of G6Pase) allowed to suggest that the gluconeogenesis, and therefore most likely the export of glucose from the liver, was also reduced in the insulin-treated trout. Altogether, these results were quite encouraging as for the first time a combined metabolic response was achieved in response to physiological doses of insulin during the postprandial period. Further, this concerned a carnivorous species reputed to badly handle the rich-carbohydrate diets. Unlike the short-term trial, the results obtained after 5 days of insulin infusion were disappointing, as we failed in obtaining normoglycaemic fish over a longer period than just one meal. Consistent with this, only minor changes were noticed in the muscle or adipose tissue (represented by an increased Akt phosphorylation level) and most of the hepatic changes explaining the reduced glycaemia after one single meal just disappeared after 5 d of high carbohydrate feeding. The only possibility rose by then was a possible loss of sensitivity due to the chronic exposure to the exogenous insulin, even though the doses infused were very low. As a whole, this study provided very encouraging results concerning the postprandial handling of carbohydrates and the insulin efficiency in a carnivorous fish species. However, several questions remained unanswered: why fish did not respond to the long-term insulin infusion? We may speculate that the continuous chronic insulin infusion could, despite the very low doses delivered, degrade the insulin signalling pathway by over-stimulation, as insulin was no longer able to stimulate Akt phosphorylation in peripheral tissues, like the muscle. Further, if exogenous insulin was efficient in lowering postprandial glucose levels, why fish do experience hyperglycaemia without pharmacological support (osmotic pump insulin delivery)? Certainly, further studies need to be conducted on the pancreatic capacity of this species to secrete the sufficient amount of insulin to handle a high carbohydrate meal. It is therefore possible that this species, naturally