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GLYCOGEN CONTENT AND ACTIVITIES OF GLYCOGEN METABOLISM ENZYMES IN CULTURED MUSCLE CELLS FROM RN- CARRIER PIGS

Marielle ESTRADE, X. VIGNON, A. TALMANT and G. MONIN
Station de Recherches sur la Viande, INRA, Saint-Genès-Champanelle, France.

OBJECTIVES

Muscle from pigs carrying the RN- allele of the rn+/RN- gene is characterized by decreased technological ability (Naveau, 1986). This defect results from an increased glycogen content, a lowered protein content and ultrastructural abnormalities (Monin et al., 1992; Estrade et al., 1993). Estrade et al. (1994) observed a marked increase in branching enzyme activity in RN- carrier pigs. There is a question if the increases in glycogen content and branching enzyme activity constitute a direct biochemical expression of the RN- allele or an indirect response to some physiological defect such as a deficiency in one or several of the hormones affecting glycogen metabolism. The aim of the present study is to answer this question using culture of muscle satellite cells, as cell culture is a mean to perform biochemical measurements in a perfectly controlled cell environment.

MATERIAL AND METHODS

ANIMALS AND SAMPLING: The glycolytic potential was determined on muscle biopsies from 25 pigs of an experimental herd including RN- carrier pigs (Talmant et al., 1989). Six RN- carrier pigs (glycolytic potential of 250-297 µmol/g fresh muscle; referred to as RN- pigs) and 6 normal pigs (glycolytic potential of 118-166 µmol/g fresh muscle) were retained for the study. The animals were slaughtered at about 100 kg liveweight in an experimental facility. Within 5 min after exsanguination, 50 g of muscle were excised from each of Longissimus lumborum, Trapezius and Masseter and immediately soaked in sterile phosphate buffer (PBS, pH 7.2) added with 100 µg/ml gentamycine.

CULTURE OF MUSCLE CELLS: Muscles were washed with sterile PBS then approximately 2 g were minced and incubated in Dubelcco's Modified Eagle's Medium (DMEM, Sigma), 10% foetal calf serum (FCS, Gibco), 0.15% pronase E (Sigma), 0.05% collagenase type V (Sigma) for 2 h at 37°C in a shaking water bath. Then the cell suspension was filtered through 4 layers of cheesecloth and centrifuged (700g for 10 min). The pellet was resuspended in DMEM with 10% FCS and 10^{-6} M dexamethasone (Sigma) and washed 3 times in this medium. Cells were seeded in Petri dishes (100 mm diameter) at a density of 10^5 cells/ml in DMEM, 20% FCS, 10^{-6} M dexamethasone and 50 µg/ml gentamycine and grown at 37°C under 95% air-5% CO2. At confluence, the cells were subcultured. When confluence was reached again, the proliferation medium was replaced by a fusion-promoting medium : DMEM with 2% horse serum, 10 µg/ml insulin and 5 µg/ml transferrin. Cultures were harvested for glycogen and enzyme analyses 7 days after the switching in this medium.

GLYCOGEN DETERMINATION: The cells layers were rinsed with cold PBS buffer then trypsinated, rinsed in PBS buffer added with 0.1 % iodoacetic acid and centrifuged (1000 g for 10 min) twice with PBS-iodoacetic acid. The pellets were finally homogenized at 4°C in the same buffer using a ground glass tissue grinder. One part of the homogenate was used for protein determination. The rest was used for glycogen determination according to Dalrymple and Hamm (1973).

ENZYME ASSAYS: The cells were treated as above, except that iodoacetate was omitted in PBS buffer. The final pellet was homogenized in buffer (63 mM glycylglycine, 500 mM saccharose, 125 mM EDTA, 125 mM NaF, pH 7.4) using a ground glass tissue grinder. Cells were stored at - 80°C until enzyme assays. Creatine phosphokinase (CPK) activity was assayed according to the technique of Oliver et al. (1955). Activities of glycogen synthase (I and I+D) , glycogen phosphorylase (a and a+b), branching enzyme and debranching enzyme were assayed as described by Briand et al. (1981).

PROTEIN DETERMINATION: The protein content of the homogenate was assessed by the method of Bradford using bovine serum albumin (BSA) as standard.

RESULTS AND DISCUSSION

Activities of CPK are reported in Figure 1. Independently of the genetic type of animals, muscle type influenced CPK activity. Creatine phosphokinase activity was higher in cells culture from Masseter than in cells from Trapezius ($P<0.05$), Longissimus being intermediate. This may be due to a different rate of satellite cells present in the muscles or to a difference in the fusion rate according to the muscle type. No significant difference was observed between RN- and normal pigs, indicating that the state of differentiation was. similar in both . This allows comparison between cells from normal and RN- pigs for the other activities.

Glycogen content was lower in cells from Masseter than in cells from both other muscles ($P<0.05$) (Figure 2). In cells from Longissimus,

glycogen content was twice higher in cells from RN- pigs than in cells from normal pigs ($P<0.05$). There was no difference between RN- and normal pigs in cells from both Masseter and Trapezius .

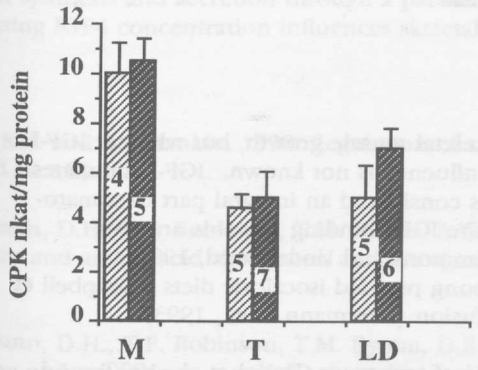

Figure 1. Activities of CPK in cultures of satellite cells from Masseter (M), Trapezius (T) and Longissimus (LD).

rn+ pigs RN- pigs
Values are means ± SEM.
The number of animals is given on the bars.

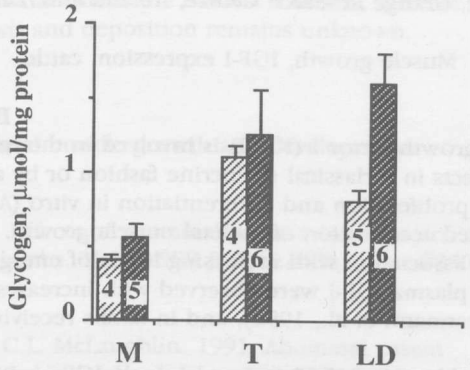

Figure 2. Glycogen level in cultures of satellite cells from Masseter (M), Trapezius (T) and Longissimus (LD).

rn+ pigs RN- pigs
Values are means ± SEM.
The number of animals is given on the bars.

Activities of glycogen metabolism enzymes in cells from Longissimus of RN- and normal pigs are reported in Table 1. There were no significant differences between cells from RN- and normal pigs concerning glycogen synthase, phosphorylase and debranching enzyme activities. By contrast, branching enzyme activity was twice higher in the cells of RN- pigs than in the cells of normal pigs ($P<0.05$).

The expression of the gene in cell cultures allows to exclude an hormonal or environmental dependency and indicates that the metabolic defect associated with the RN- gene is localized in the muscle cell. This observation confirms the conclusion of Monin et al. (1992) that the increase in muscle glycogen content is not secondary to an hormonal deficiency.

TABLE 1. Activities of glycogen metabolism enzymes in cultured satellite cells of Longissimus from normal and RN- carrier pigs.

Enzymes	normal pigs (n = 5)	RN- pigs (n = 6)	Significance
Phosphorylase a, nkat/mg protein	0.06 ± 0.02	0.04 ± 0.01	
Phosphorylase a+b, nkat/mg protein	0.18 ± 0.04	0.10 ± 0.02	
Debranching enzyme, pmol glucose/s/mg protein	0.59 ± 0.05	0.68 ± 0.08	
Glycogen synthase I, nkat/mg protein	0.08 ± 0.02	0.06 ± 0.02	
Glycogen synthase I+D, nkat/mg protein	0.36 ± 0.08	0.21 ± 0.04	
Branching enzyme, nmol/s/mg protein	9.8 ± 0.3	15.2 ± 1.6	$P < 0.05$

CONCLUSION

The results confirm that the RN- gene increases the glycogen level and the branching enzyme activity in muscle. They allow to conclude that the primary metabolic defect associated with the RN- gene is localized in the myofibers.

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