Studies on Exercise and Glycogen Re-synthesis in Skeletal Muscles of Pigs with the *PRKAG3* Mutation

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Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2011

Acta Universitatis agriculturae Sueciae
2011:56

Cover: Bonnie, one of the participants who made this thesis possible (photo: A. Granlund)

ISSN 1652-6880
ISBN 978-91-576-7600-9

Print: SLU Service/Repro, Uppsala 2011

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Abstract

AMP-activated protein kinase (AMPK) plays an important role in the regulation of glucose and lipid metabolism in skeletal muscle. Many pigs of Hampshire origin have a naturally occurring mutation situated in the PRKAG3 gene which encodes a muscle-specific isoform of the AMPK γ3-subunit. This mutation results in excessive glycogen storage mainly in white glycolytic skeletal muscle and an increased muscle oxidative capacity.

This thesis examined the *in vivo* effect of the *PRKAG3* mutation on skeletal muscles in association with exercise. The pigs performed standardised treadmill exercise tests before and after a 5-week training period. Muscle samples (*m. biceps femoris*) and blood samples were taken before and after exercise and in the recovery phase. Muscle samples from *m. masseter*, *m. biceps femoris, m. semitendinosus* and *m. longissimus dorsi* were taken after euthanasia. Glycogen content and its two fractions (proglycogen and macroglycogen), enzyme activity, fibre type characteristics and expression/phosphorylation of signalling proteins were analysed. Insulin, glucose, lactate and free fatty acid concentrations were analysed on the blood samples.

Compared with non-carriers, carriers of the *PRKAG3* mutation had higher macroglycogen content in skeletal muscles. At 3 h but not 6 h of recovery following exercise, re-synthesis of glycogen, especially of macroglycogen was higher in carriers than in non-carriers. The metabolic blood response did not differ between the genotypes. Expression of AMPK in muscle was lower in carriers than in non-carriers. The increased rate of glycogen synthesis following exercise in carriers was correlated with an increased signalling response of Akt and its substrate AS160 and a higher activity of hexokinase. This indicated increased glucose influx and phosphorylation of glucose, directed towards glycogen synthesis. The carriers had a lower percentage and relative area of type IIB fibres in *m. biceps femoris* and *m. longissimus dorsi* than non-carriers. In *m. longissimus dorsi,* carriers had a higher percentage of type IIAX fibres and higher citrate synthase activity. In conclusion, the *PRKAG3* mutation influences, mainly the macroglycogen fraction and muscle characteristics in exercise-trained pigs, promoting varying degrees of oxidative phenotype in muscles with different functions.

Keywords: pig, glycogen, skeletal muscle, RN mutation, AMPK, fibre type

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Dedication

To Johan, Karl, Gustav, Nils and Elsa.

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Granlund, A., Kotova, O., Benziane, B., Galuska, D., Jensen-Waern, M., Chibalin, A.V. & Essén-Gustavsson, B. (2010) Effects of exercise on muscle glycogen synthesis signalling and enzyme activities in pigs carrying the *PRKAG3* mutation. *Exp Physiol* 95, 541-549.

II Essén-Gustavsson, B., Granlund, A., Benziane, B., Jensen-Waern, M. & Chibalin, A.V. (2011). Muscle glycogen re-synthesis, signalling and metabolic responses following acute exercise in exercise-trained pigs carrying the *PRKAG3* mutation. Submitted.

III Granlund, A., Jensen-Waern, M. & Essén-Gustavsson, B. (2011). The influence of the *PRKAG3* mutation on glycogen, enzyme activities and fibre types in different skeletal muscles of exercise trained pigs. *Acta Vet Scand* 24, 53-20.

IV Granlund, A., Jensen-Waern, M. & Essén-Gustavsson, B. (2011). The effect of exercise on proglycogen and macroglycogen re-synthesis in skeletal muscles in pigs carrying the *PRKAG3* mutation. In manuscript.

Abbreviations

ACC	Acetyl-CoA carboxylase
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
AS160	Akt substrate of 160 kD
ATP	Adenosine triphosphate
CS	Citrate synthase
GLUT	Glucose transporter
G-6-P	Glucose-6-phosphate
GS	Glycogen synthase
GSK-3	Glycogen synthase kinase 3
HAD	3-OH-acyl-CoA-dehydrogenase
HK	Hexokinase
LDH	Lactate dehydrogenase
MHC	Myosin heavy chain
PFK	Phosphofructokinase
RN	Rendement Napole
UDP	Uridine diphosphate

1 Introduction

Strong selection for meat quality and quantity has increased the frequency of several mutations in the pig (Fujii *et al.*, 1991; Jeon *et al.*, 1999). One of these is the mutation in the RN gene, which strongly influences the functional and sensory properties of meat. "Rendement Napole" (RN), from the French for Napole yield, is a method for estimating the yield of cured cooked ham. The existence of a gene affecting Napole yield was first suggested by the French meat scientist Naveau (Naveau *et al.*, 1985) and later confirmed by Le Roy *et al.*, (1990). The dominant RN^- allele increases the glycogen content of pig muscles (Monin & Sellier, 1985; Naveau *et al.*, 1985), but the glycogen content in heart and liver is unaffected (Monin *et al.*, 1992). The RN^- allele has only been found in Hampshire pigs and it is likely that the mutation arose in this breed and has increased in frequency due to its favourable effects on growth rate and meat content in the carcass (Enfält *et al.*, 1997). Meat from RN^- pigs has a low ultimate pH (because of post-mortem degradation of the excess glycogen), a reduced water-holding capacity and results in a reduced yield of cured cooked ham (Estrade *et al.*, 1993; Enfält *et al.*, 1997). The muscle of RN^- carriers has a higher water content and this is probably why the RN^- pigs are classified as having higher lean meat content (Enfält *et al.*, 1997; Le Roy *et al.*, 2000). Lean meat content is the proportion of muscle in the total carcass weight of a pig, which has been an important selection goal in pig breeding over the last 50 years.

No pathological effects of the RN^- mutation have been reported and it does not cause glycogen storage disease. Muscle fibres from RN^- carrier pigs show up to 70% excess glycogen in both sarcoplasmic and lysosomal compartments compared with non-carriers (Estrade *et al.*, 1993). The RN^- mutation has been mapped to chromosome 15 (Milan *et al.*, 1995; Mariani *et al.*, 1996). The causative mutation has been characterised as a single

nucleotide substitution in the PRKAG3 gene, resulting in the non-conservative arginine to glutamine substitution at amino acid position 220 (Milan *et al.*, 2000). A second mutation (V224I) at the neighbouring amino residue has also been identified in pigs and is associated with an opposite effect, low glycogen and high pH compared with the RN^- allele (Ciobanu *et al.*, 2001). The PRKAG3 gene encodes a muscle-specific isoform of the regulatory γ3-subunit of adenosine monophosphate-activated protein kinase (AMPK) (Milan *et al.*, 2000). AMPK is a fuel sensing enzyme that is present in most mammalian tissues and responds to shifts in cellular energy levels. When activated, AMPK inhibits ATP-consuming pathways and stimulates ATP-generating pathways (Hardie *et al.*, 1997). It is known to inhibit enzymes involved in the synthesis of glycogen, fatty acid and cholesterol and to stimulate glucose uptake, glycolysis and fatty acid oxidation (Kemp *et al.*, 1999; Ojuka *et al.*, 2000). As AMPK is a key metabolic enzyme, the *PRKAG3* mutation is expected to influence the regulation of several key metabolic processes in the cell. Contraction or exercise increases the use of energy in skeletal muscle, which leads to increase in AMP: ATP ratio and activates AMPK.

The pig is an important animal model in many areas of biomedical research due to its anatomic and physiological similarities to man (Swindle *et al.,* 1988; Almond, 1996). For instance pigs have played a role in exercise physiology for over 40 years (Rülcker, 1968). The pigs used in this thesis, non-carriers and carriers of the *PRKAG3* mutation, were subjected to acute and chronic exercise in order to develop a new animal model for studies of carbohydrate metabolism. Exercise increases the utilisation of the glycogen stores within the working muscle and stimulates AMPK. AMPK is considered an important mediator in insulin-independent glucose transport in skeletal muscle (Fujii *et al.*, 2000). Thus, using pigs that differed markedly in glycogen content before exercise and sampling of blood and muscle tissues in connection with exercise allowed detailed studies of the interaction between glycogen stores in skeletal muscle and glucose metabolism. Another aspect investigated was whether the carriers of the *PRKAG3* mutation with high glycogen content in their muscles, had enhanced muscle ergogenics, since increased glycogen stores in muscle are associated with increased exercise capacity in man (Bergström *et al.*, 1967). Glycogen in skeletal muscle contains two different fractions, smaller acid-insoluble proglycogen particles and larger acid-soluble macroglycogen particles (Lomako *et al.*, 1991). A previous study, in which only a few pigs were included, showed faster re-synthesis of glycogen, especially of macroglycogen, in the carriers after exercise (Essén-Gustavsson *et al.,* 2005).

This thesis therefore included a larger number of pigs and examined additional factors that may influence glycogen re-synthesis after exercise.
Since the *PRKAG3* mutation was discovered due to meat quality studies, most research in this area has concentrated on the cutlet, *m. longissimus dorsi*. This thesis therefore extended the research area by investigating the effect of the mutation in some muscles with different functions. *M. biceps femoris,* a skeletal muscle which has been shown to be active during treadmill exercise, was one of the muscles investigated.

The *PRKAG3* mutation in pigs is associated with an almost twofold increase in glycogen content in skeletal muscle. However, the mechanisms for the high capacity to store glucose as glycogen in these pigs are unknown. This thesis therefore examined factors associated with the increased glycogen storage in skeletal muscle in pigs with the *PRKAG3* mutation.

1.1 Background

1.1.1 AMPK

AMPK is a heterotrimeric complex that consists of a catalytic α-subunit and regulatory β- and γ-subunits, which are highly conserved in almost all eukaryotes (Hardie *et al.*, 1998). Both the α and β-subunits are encoded by two genes (α1, α2, β1, β2) and the γ-subunit is encoded by three genes (γ1, γ2, γ3). AMPK is expressed in all mammalian tissue, with some tissue-specific isoform expression of the regulatory β-and γ-subunits.

The regulatory γ3-subunit is the main isoform expressed in skeletal muscle, particularly fast-glycolytic muscle (Mahlapuu *et al.*, 2004). In contrast, expression of the γ1-and γ2-subunits is widely distributed in tissues. The γ-subunit stabilises the catalytic α-subunit of AMPK and is necessary for the catalytic activity of AMPK in mammals. Apart from the mutations in the γ3 isoform in pigs several mutations have been identified within the γ2 isoform in man that cause cardiac hypertrophy, supraventrical tachycardia and elevated cardiac glycogen storage associated with Wolff-Parkinson-White syndrome (Blair *et al.*, 2001; Daniel & Carling, 2002).

1.1.2 Glycogen

Glycogen is a glycoprotein with elongated and branched polysaccharide chains. The glycogen part is built in tiers around a central protein molecule

known as glycogenin. The glycogen granule also contains the proteins required for glycogen metabolism. Therefore the glycogen granule is not merely a storage site for carbohydrates, but also an independent organelle-like structure referred to as the glycosome (Rybicka, 1996).

The primary function of glycogen is to provide storage for glucose and this takes place mainly in the liver and skeletal muscle. The highest concentration of glycogen is found in the liver, but since the skeletal muscle has a greater mass, it contains more glycogen. Glycogen can be rapidly broken down to control blood glucose level in response to fasting or muscle contraction. The energy requirements of the cell, previous glycogen content, enzymatic activity and levels of expression/phosphorylation of signalling proteins, hormonal stimulation and training status are factors that influence how much glucose enters glycogenesis. Once inside the cell, glucose is rapidly phosphorylated by hexokinase to form glucose-6-phosphate and then glucose-1-phosphate is formed by the enzyme phosphoglucomutase (Figure 1). The biosynthesis of glycogen involves a specific initiation phase since glycogen synthase (GS) can only lengthen an existing chain (Manchester *et al.*, 1996). Glycogen synthesis starts with uridine-triphosphate (UTP) which reacts with glucose-1-phosphate to form uridine-diphosphate glucose (UDP-glucose), a step which is catalysed by UDP-glucose pyrophosphorylase. Glycogenin, a self-glycosylating and autocatalytic protein then catalyses the addition of a single UDP-glucose by a C-1-O tyrosyl linkage at amino acid residue Tyr-194. Thereafter glycogenin adds additional UDP-glucose residues to the single glucose residue to form an oligosaccharide chain of 8-12 glucosyl units. The "primed" glycogenin then works as a substrate for GS and branching enzyme (the elongation phase), which extend the oligosaccharide chain to form the characteristic branched structure (Lomako *et al.*, 1988; Alonso *et al.*, 1995). There are two types of bonds that link glucose units together: α-1,4 linkages join glucose units in straight linkages, account for 93% of all bonds and are formed by GS; while α-1,6 branched linkages account for the remaining 7% of bonds and are formed by branching enzyme. At predicted maximal size, the glycogen molecule has a diameter of 42 nm and contains 55000 glucose residues in 12 tiers (Goldsmith *et al.*, 1982). The high density of glucose on the outermost tier prevents the addition of more glucose by GS (Melendez *et al.*, 1997).

Glycogenolysis is catalysed by the enzyme glycogen phosphorylase, which cleaves α-1,4 glucosyl bonds to form glucose-1-P. Branches have to be removed by debranching enzyme, since phosphorylase can only cleave off glucosyl units that are joined in a linear way. Muscle glycogen content is

an important energy source that is used during physical exercise and high glycogen content is associated with optimal performance in man (Bergström, 1967). Decrease in muscle glycogen content during exercise is dependent on factors such as exercise intensity, type of exercise and duration (Holloszy *et al.*, 1998).

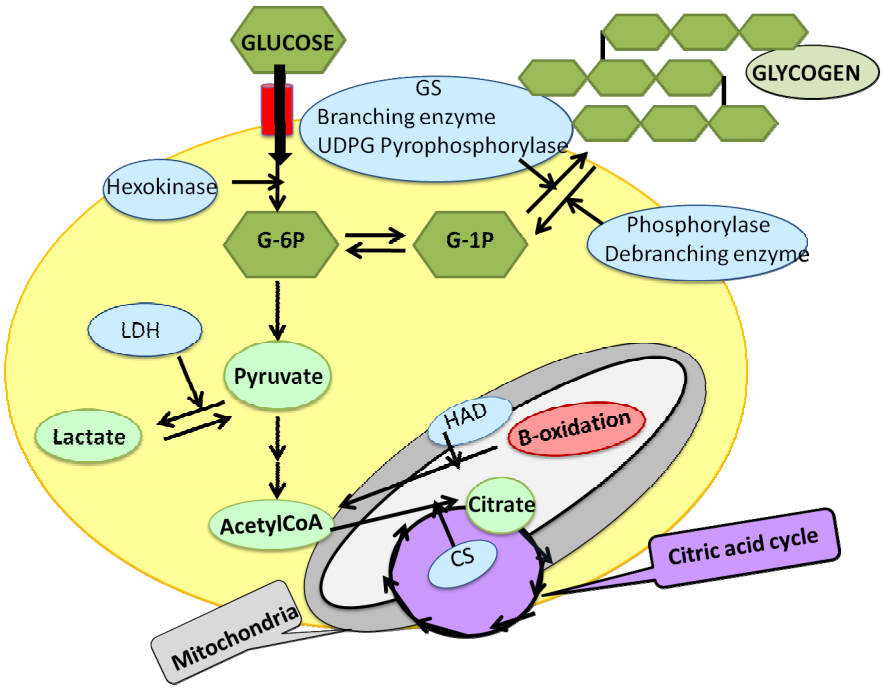

Figure 1. Intracellular fate of glucose.

A study has shown that branching enzyme activity is twice as high in the muscle of carriers of the *PRKAG3* mutation, but no differences have been observed between carriers and non-carriers concerning GS, phosphorylase or debranching enzyme (Estrade *et al.*, 1994). Proteome analyses of *m. longissimus dorsi* in pigs, have shown that an enzyme used in the synthesis of glycogen, UDP-glucose pyrophosphorylase, is significantly up-regulated in *PRKAG3* carriers. The mRNA transcription and activity of UDP-glucose pyrophosphorylase is also up-regulated in carriers (Hedegaard *et al.*, 2004).

1.1.3 Proglycogen and macroglycogen

In recent years it has been suggested that the well-known glycogen synthesis model with glycogenin, glycogen synthase, branching enzyme and

the substrate UDP-glucose is not adequate to explain glycogen synthesis. Instead, a theory claims that glycogen exists in two forms, the smaller proglycogen with a molecular weight of 400 kDa and the larger macroglycogen with a molecular weight of 10000 kDa (Lomako *et al.*, 1991; Lomako *et al.*, 1993; Alonso *et al.*, 1995). These two fractions of glycogen are characterised by their number of glucose units and their solubility in acid. The synthesis of glycogen from glycogenin to mature glycogen via the formation of proglycogen is suggested to be catalysed by three different enzyme activities. The first phase in glycogen synthesis is self-glucosylation by glycogenin. Thereafter, proglycogen is formed by proglycogen synthase and finally macroglycogen is synthesised by macroglycogen synthase (Figure 2). Whether proglycogen and macroglycogen are actually distinctly different kinds of glycosomes or whether they simply differ on the basis of the size of the actual glycogen molecule is unclear (Marchand *et al.*, 2002; Shearer & Graham, 2004). Acid separation of glycogen into two fractions is of use to separate smaller and larger glycogen granules, which is of significance since the size of the glycogen granules has an impact on the rate of synthesis, degradation and also sensitivity to carbohydrate supplementation (Adamo *et al.*, 1998; Graham *et al.*, 2001; Battram *et al.*, 2004). Interestingly, the macroglycogen fraction in humans and rats is reported to increase with high muscle glycogen concentrations (Jansson *et al.*, 1981; Derave *et al.*, 2000). The re-synthesis of glycogen has been shown to occur mainly in the macroglycogen pool in man, when the total glycogen concentration in the muscle reaches a concentration of over 300-350 mmol glucosyl units/kg dw (Jansson *et al.*, 1981; Adamo *et al.*, 1998). A previous study of few pigs with the *PRKAG3* mutation indicated that the carriers had higher macroglycogen stores in *m. biceps femoris* at rest than the non-carriers. Glycogen degradation during treadmill exercise did not differ between the genotypes, but the re-synthesis of macroglycogen was faster in the early recovery period in the carriers (Essén-Gustavsson *et al.*, 2005). Exercise performance on a treadmill was not affected by the high glycogen content in the carriers.

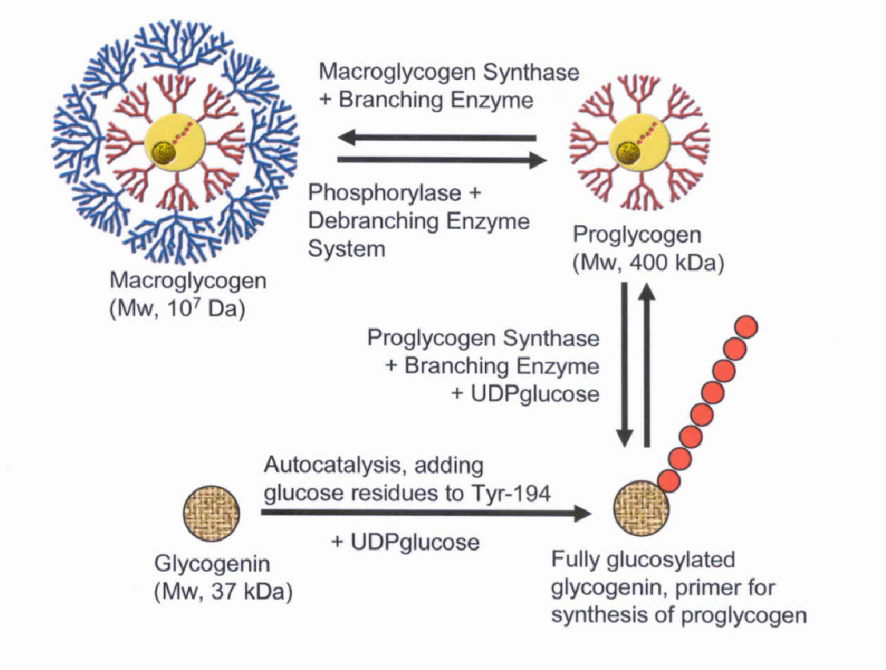

Figure 2. The proposed model of biogenesis of glycogen. Glycogenin autocatalytically adds glucose from UDP-glucose to its tyr-194 and then an average of 7 residues to form a fully glucosylated glycogenin that serves as a primer for the synthesis of proglycogen by proglycogen synthase and branching enzyme. Proglycogen was suggested to be a stable intermediate in the synthesis of mature glycogen (Macroglycogen). The priming chain of 8 glucose residues corresponds to the average length of elongation but in actuality ranges from 7 to 11 glucose residues (Lomako et al. 1993).

1.1.4 Glucose metabolism

Glycogen is one of the major fuel sources used during exercise and in order to be able to re-synthesise glycogen, glucose is needed. Glucose is taken up from the blood but cannot penetrate the lipid bilayer without a carrier protein (due to the hydrophilic nature of glucose). A total of 12 facilitative transporters (GLUT1-12) have been identified (Joost *et al.*, 2002) and in skeletal muscle insulin-sensitive GLUT4 is the major transporter (Birnbaum, 1989). GLUT4 translocates from an intracellular compartment to the cell surface in response to either insulin or exercise (Douen *et al.*, 1990).

Insulin signalling

Insulin-sensitive tissues express insulin receptors at the cell surface, which allows them to regulate metabolism through a specific insulin signalling

cascade. The insulin signalling events start by insulin binding to the insulin receptor (IR) and autophosphorylation of IR tyrosine kinase, causing tyrosine phosphorylation of insulin receptor substrates (IRS) 1-4 (both IRS-1 and IRS-2 are expressed in skeletal muscle) and activation of phosphatidylinositol 3-kinase (Alessi *et al.*, 1997; White, 1998). One of the downstream pathways by which insulin-stimulated phosphatidylinositol 3-kinase (PI 3K) activity leads to GLUT4 translocation involves activation of the serine/threonine kinase PKB/Akt. There is evidence that Akt functions downstream of PI 3K (Kohn *et al.*, 1996) and mediates insulin signalling to glucose transport (Cho *et al.*, 2001). At present there are three known isoforms of Akt (Akt 1-3) but only Akt 1 and Akt 2 are expressed in skeletal muscle (Cho *et al.*, 2001). It has been shown that Akt 1 and Akt 2 have different downstream metabolic regulation, in that Akt 1, together with IRS-2, is important for lipid metabolism, while Akt 2 and IRS-1 are essential for glucose metabolism (Bousakri *et al.*, 2006). Once activated, Akt phosphorylates downstream substrates such as the Akt substrate of 160 kDa (AS 160). AS 160 is activated and redistributed to the plasma membrane following phosphorylation of Akt (Kane *et al.*, 2002). In adipocytes AS160 mediates insulin-stimulated Akt signalling to glucose transport probably by regulation of GLUT4 endocytosis (Sano *et al.*, 2003). AS 160 is also expressed in the rat skeletal muscle and phosphorylated in response to insulin and contraction (Bruss *et al.*, 2005). In addition to glucose transport Akt mediates the effect of insulin on glycogen synthesis via glycogen synthase kinase 3 (GSK-3) phosphorylation, which decreases its inhibitory effect on glycogen synthase (Cross *et al.*, 1995). This leads to increased glycogen synthesis (Figure 3).

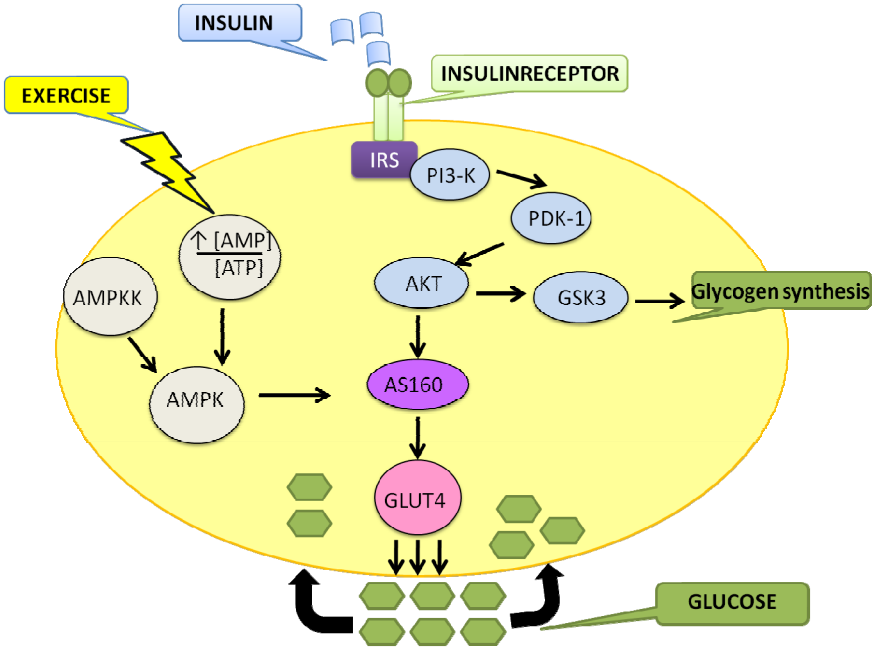

Figure 3. Insulin- and exercise signalling mechanisms to stimulate glucose transport involve GLUT4 translocation.

Exercise signalling

Exercise or muscle contraction can also promote glucose transport in skeletal muscle (Holloszy & Kohrt, 1996) but the mechanism is less well documented than the insulin signalling mechanism. Several studies have shown that insulin- and exercise-induced glucose uptake is activated through two separate pathways. There is evidence that a maximally effective insulin concentration in combination with contractile activity stimulates glucose transport (Zorzano *et al.*, 1986; Wallberg-Henriksson *et al.*, 1988), and GLUT4 translocation (Lund *et al.*, 1995) to a greater degree than either stimulus alone and that the times when glucose uptake occurs are different (Wallberg-Henriksson *et al.*, 1987). Wortmannin, a PI 3-kinase inhibitor, prevents insulin-mediated, but not contraction-mediated glucose transport (Lee *et al.*, 1995) and translocation of GLUT4 (Lund *et al.*, 1995) in isolated skeletal muscle. Exercise and insulin-sensitive GLUT4 pools may exist (Douen *et al.*, 1990). Exercise/muscle contraction is not sufficient to activate IR (Goodyear *et al.*, 1995), IRS1 (Goodyear *et al.*, 1995) or Akt (Brozinick & Birnbaum, 1998; Widegren *et al.*, 1998). Recent studies have suggested that the two signalling pathways may converge at the point of AS160 and that their effects can be added (Kramer *et al.*, 2006). A single session of

exercise and also exercise training result in improved insulin sensitivity in skeletal muscle (Richter *et al.*, 1982; Wallberg-Henriksson *et al.*, 1987). A number of exercise-responsive molecules have been proposed as possible candidates for exercise-responsive glucose uptake including nitric oxide (Roberts *et al.*, 1999), Ca^{2+}/calmodulin-dependent protein kinases (Wright *et al.*, 2004) and AMPK (Fujii *et al.*, 2000).

Intracellular fate of glucose

Once glucose has entered the cell it is rapidly phosphorylated to glucose 6-phosphate (G-6-P) by hexokinase (HK) and then stays trapped. G-6-P can either be converted to glycogen or transferred in the glycolysis pathway to pyruvate. The fate of glucose is mainly determined by the energy requirement of the cell. ATP and creatine phosphate are the most rapidly accessible energy fuels. Muscle glycogen, triglycerides, blood-borne glucose and fatty acids are other energy resources. Fatty acids are metabolised to acetyl-CoA by mitochondrial enzymes responsible for beta-oxidation, one of which is 3-OH-acyl-CoA-dehydrogenase (HAD). For short-term exercise the muscle cells rapidly metabolise glycogen to pyruvate (glycolysis). Pyruvate can either be metabolised aerobically in the citric acid cycle where citrate synthase (CS) is the first key enzyme or anaerobically to lactate, by lactate dehydrogenase (LDH). The muscle cell can shuttle the lactate into a neighbouring fibre within the same muscle or into the blood, where it can be used as a fuel by the heart or another tissue. Lactate is also an important precursor to gluconeogenesis in the liver. Aerobic metabolism is sufficient to meet the energy demand when oxygen is adequate and the muscle is working with low intensity. If contraction proceeds faster and with high intensity, oxygen becomes limiting and then the muscle has to use the rapid anaerobic glycolytic pathway to supply energy.

Studies of carriers of the *PRKAG3* mutation have shown that they have increased oxidative capacity in the *m. longissimus dorsi*. (indicated by higher CS activity) (Estrade *et al.*, 1994; Lebret *et al.*, 1999) and decreased glycolytic capacity (indicated by lower LDH activity) (Lebret *et al.*, 1999). No significant differences have been found between heterozygous and homozygous carriers of the RN^- allele except for the observation that the CS activity is lower in the RN^-/rn^+ than in the RN^-/RN^- (Lebret *et al.*, 1999). The expression patterns of enzymes related to glycolysis and the citric acid cycle, such as phosphofructokinase (PFK), the β-subunit of ATP-specific succinyl-CoA synthetase and mitochondrial aldehyde dehydrogenase, have also been shown to be up-regulated by the mutation in *m. longissimus dorsi* of pigs (Hedegaard *et al.*, 2005).

1.1.5 Skeletal muscle fibre characteristics

Skeletal muscle is the largest tissue in the body and is responsible for most types of body movement. Over 90% of the muscle consists of the muscle fibres. The muscle cell is a multinucleated elongated cell (fibre), and its diameter varies both within and between muscles, the size being affected by growth and activity. The fibre can contract since it is innervated by neurons from the spinal cord. Different histochemical staining techniques can be applied to visualise biochemical properties such as enzyme, glycogen and lipid levels in individual muscle fibres (Figure 4).

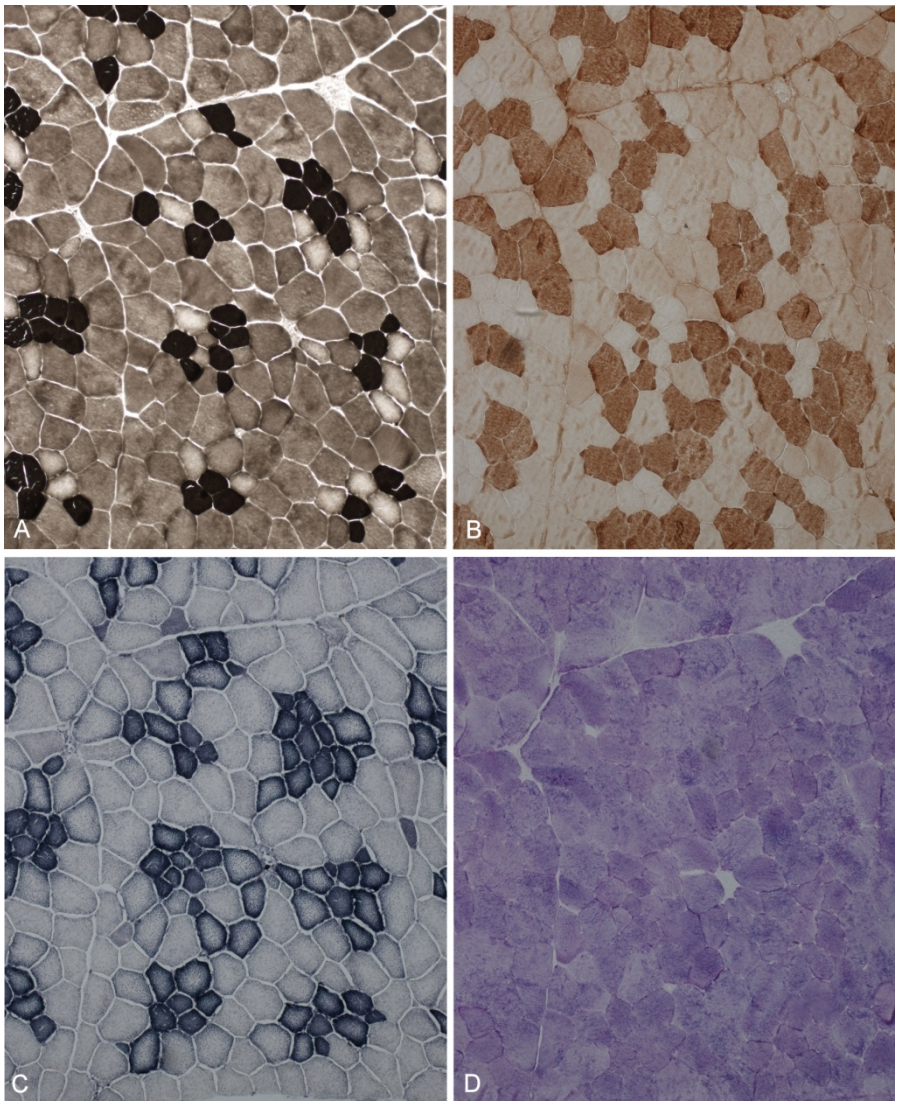

Figure 4. Histochemical stains for **A**. myosin ATPase after preincubation at 4.6, **B**. Immunohistochemical stains for A4-74, **C.** NADH-tetrazolium reductase, **D**. PAS. The muscle sample is obtained from *m. biceps femoris* of a non-carrier pig.

Through such methods, it has been demonstrated that skeletal muscle is composed of a mixture of fibre types with different contractile and metabolic properties. In most animals, glycogen is found at rest in all fibres, but glycogen can decrease within the fibres depending on the recruitment of the fibres with exercise. Fibre type composition and the physical activity level of the muscle are factors that may contribute to the differences seen in glycogen content and enzyme activity between muscles. Most skeletal

muscles contain a mixture of different fibre types but in certain species e.g. rodents, individual muscles mostly consist of one particular fibre type. Fibre types are commonly divided into two major groups; slow-twitch (type I) and fast twitch (type II). Type II fibres can further be sub grouped into type IIA and IIB fibres by using myosin ATPase staining technique following acid and alkali preincubations (Brooke & Kaiser, 1970). Myosin ATPase staining studies have revealed that muscles of the pig mostly show an organised pattern and unique distribution of different fibre types consisting of islets of type I fibres immediately surrounded by type IIA and more peripherally by type IIB fibres (Figure 4). The fibres contain different myosin heavy chains isoforms that are responsible for their different myosin ATPase activities and speeds of contraction. Type I fibres are usually involved in low intensity training and rely on oxidative metabolism of lipids and carbohydrates for their energy supply. Type II fibres are recruited in more intense exercise and depend to a great extent on utilisation of carbohydrate and lactate. Another method of characterising fibre types is to use myosin heavy chain (MHC) antibodies that identify the different MHC isoforms MHC I, MHC IIA MHC IIX and MHC IIB. Immunohistochemistry makes it possible to detect hybrid fibres, i.e. fibres that contain more than one MHC isoform, which may be an indication that fibres are under transformation. Type II fibres can be divided into three classes depending on their different MHC isoforms; IIA, IIB and IIX, where type IIA is fast fatigue resistant, IIB is fast fatigable, and IIX elicits an intermediate fatigue response (Schiaffino *et al.*, 1989). The classification is different in different species. The IIB isoform was previously only considered to be present in small animals such as mouse, rat, guinea pig and rabbit (Bär & Pette, 1989; Schiaffino *et al.*, 1989). However more recent studies have shown that pigs also exhibit IIB fibres, mostly in their glycolytic muscles (Lefaucheur *et al.*, 1998; Lefaucheur *et al.*, 2002; Tonioli *et al.*, 2004). Myosin heavy chains can be transformed following a compulsory pathway: I↔IIA↔IIAX↔IIX↔IIB, although the shift is reversible (Schiaffino & Reggiano, 1994; Pette & Staron, 2000). The pig is a meat producing animal, which has been selected and fed to increase muscle development, and this has resulted in a fibre-type shift toward type II (Solomon & West, 1985; Weiler *et al.*, 1995). In contrast, endurance exercise training has been shown to shift the fibre type towards type I in rats (Green *et al.*, 1984) and in man (Baumann *et al.*, 1987). Studies in pigs also indicate that training may cause fibre-type shifts from type IIB (ATPase method) to IIA (Essén-Gustavsson *et al.*, 1993; Petersen *et al.*, 1998). Oxidative capacity is known to increase with training and among fibre types oxidative metabolism is high in type I fibres and decreases in the order type I > type IIA > type IIX > type IIB fibres (Lefaucheur *et al.*, 2002). Interestingly wild boars have been shown to have higher oxidative capacity,

a higher proportion of type IIA fibres and larger mean fibre area compared with domestic pigs (Karlström, 1995)

It has previously been shown that the *m. longissimus dorsi* is more oxidative in pigs with the *PRKAG3* mutation, as indicated by increased CS and HAD activity, decreased LDH activity, higher relative area of oxidative type IIA fibres and lower relative area of non-oxidative type IIB fibres (Lebret *et al.*, 1999). In contrast in *m. semispinalis capitis* (mostly type I fibres), there is no influence of the *PRKAG3* mutation on enzyme activity.

1.1.6 Exercise response and adaptation

Different types of exercise are associated with a dissimilar response and adaptation in different muscles. This is related to the fact that skeletal muscles consist of different fibre types with different metabolic characteristics and fibre recruitment patterns. The effects of exercise on muscle characteristics are also specific to the frequency and duration of the exercise performed. This makes it often difficult to compare exercise studies since they often differ in one of these aspects. In the present study, pigs performed a standardised exercise protocol by trotting on a large animal treadmill. This type of endurance training has previously been shown to affect *m. biceps femoris*, generating increased glycogen content and improved oxidative capacity (Essén-Gustavsson *et al.*, 1988) whereas no alterations were observed in *m. longissimus dorsi* and *m. semitendinosus* (Essén-Gustavsson & Lindholm, 1983; Essén-Gustavsson *et al.*, 1988). *M. longissimus dorsi* contains a large proportion of type II fibres especially type IIB fibres (80-90%) and has a low oxidative capacity (Essén-Gustavsson *et al.*, 1980; Karlsson *et al.*, 1993). The white portion of *m. semitendinosus* shares many fibre characteristics with *m. longissimus dorsi*, i.e. a high proportion of type IIB fibres and low oxidative capacity (Essén-Gustavsson *et al.*, 1980). *M. biceps femoris* has a lower proportion of type IIB fibres (60-70%) and a higher oxidative capacity (Essén-Gustavsson *et al.*, 1980; Karlsson *et al.*, 1993). *M. masseter* is a muscle which is mainly active during the chewing process and has a high proportion of type I fibres (around 90%), almost no type IIB fibres and a high oxidative capacity (Karlström, 1995).

2 Aims of the thesis

The overall aim of this thesis was to study the effect of exercise on skeletal muscles in pigs with the *PRKAG3* mutation, especially the glycogen degradation and re-synthesis.

Specific aims were:

- To study the *in vivo* effect of the *PRKAG3* mutation on the expression and phosphorylation of proteins involved in insulin and/or contraction induced signalling pathways in skeletal muscle. This was to be investigated at rest, after acute exercise, in the recovery phase and after a training period.
- To study the *in vivo* effect of the *PRKAG3* mutation on metabolic blood parameters at rest, after acute exercise and in the recovery phase in trained pigs.
- To study the *in vivo* effect of the *PRKAG3* mutation on muscle characteristics after a training period.
- To study the *in vivo* effect of the *PRKAG3* mutation on proglycogen and macroglycogen stores in skeletal muscle at rest, after acute exercise, in the recovery phase and after a training period.

3 Aspects of materials and methods

A brief presentation of the materials and methods used in Papers I-IV is given here (Figure 5). A complete description is presented in the individual papers.

3.1 Animals

Sixteen clinically healthy female pigs (Yorkshire/Swedish Landrace x Hampshire) from the SLU university herd were used. The pigs were 9-11 weeks old, had a mean weight of 29 ± 2.5 kg when they arrived, and were stalled at the Department of Clinical Sciences, SLU. Eight pigs were heterozygous carriers and eight pigs were non-carriers of the *PRKAG3* mutation, as verified by DNA analyses of blood (Milan *et al.*, 2000). The pigs were housed in pens (4 pigs/pen) with a concrete floor with straw as bedding. The animals were fed a commercial finisher diet without growth promoters (Piggfor®; Origio 522 PK, Lantmännen, Uppsala, Sweden) *ad lib* and had free access to water. The pigs were inspected daily for their clinical state of health. The experiments were approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden.

3.2 Study design

The studies were conducted during a nine-week period and started with a two-week period of acclimatisation. During this period the pigs were allowed to walk and trot on a large animal treadmill (Säto, Knivsta, Sweden) for a few minutes on four successive days. After acclimatisation, the pigs were anaesthetised and muscle biopsy specimens were taken from the

middle part of the right *m. biceps femoris* at a depth of 3-4 cm using a needle biopsy technique (Bergström, 1962) (Papers I, IV). The muscle specimens were immediately frozen in liquid nitrogen and stored at -80° C until analysis. Anaesthesia was achieved by administration of a combination of medetomidine (Domitor®vet 1mg/mL; Orion Pharma AB, Stockholm, Sweden) and tiletamine and zolazepam (Zoletil®; 250 mg tiletamine + 250 mg zolazepam/mL; Reading, Carros, France). The final mixture contained 1 mg/mL medetomidine and 100 mg/mL tiletamine-zolazepam and was given at a dosage of 0.05 mL/kg bw.

Three days after the resting biopsies had been taken, all pigs ran 700 m on the treadmill at a speed of 1.5 m/s. Within 10 minutes of completion of exercise, the anaesthetic mepivacaine (Carbocain® adrenaline 20mg/mL + 5µg/mL; Astra Zeneca AB, Södertälje, Sweden) was administered locally and a biopsy specimen was obtained from the left *m. biceps femoris* (Paper I, IV). The pigs were restrained at the front of the treadmill using a board to enable biopsy specimens to be collected. After three hours of recovery, a new biopsy from right the *m. biceps femoris* was collected under anaesthesia (see above) (Papers I, IV).

The pigs were then trained on the treadmill once daily, five days a week for the next five weeks. The speed was gradually increased from 1.5 m/s to 2.5 m/s and the distance from 300 m to 1000 m. A distance test was performed where all pigs were given the opportunity to trot on the treadmill until fatigue in the last week of training (week 8). The pigs ran 0-200 m at a speed of 2.1 m/s, 200-400 m at a speed of 2.3 m/s and 400 m to an individual terminal point at 2.5 m/s (Paper IV). Finally, the training period ended with a second exercise test where the pigs ran 1000 m on the treadmill (0 to 200 m at a speed of 2.1 m/s, 200 to 400 m at a speed of 2.3 m/s and 400 to 1000 m at a speed of 2.5 m/s). Muscle biopsy specimens were collected (after application of local anaesthetic) from the left *m. biceps femoris* within 10 minutes of completion of exercise (Paper IV). After 3 hours of recovery the pigs were anaesthetised and muscle tissue samples were obtained from the right *m. biceps femoris* (see above) (Papers IV).

Thereafter the pigs had an indwelling catheter inserted into the jugular vein under general anaesthesia, in order to facilitate frequent blood sampling. Fifteen minutes prior to induction of anaesthesia, atropine sulphate (Atropin®; NM Pharma AB, Stockholm, Sweden) was given i.m. at a dosage of 0.05 mg/kg bw. Anaesthesia was then induced with a combination of medetomidine and tiletamine/zolazepam at a dosage of 0.05 mL/kg bw. The animals were then intubated and general anaesthesia was maintained by isoflurane, with oxygen and nitrogen monoxide. The surgical procedure has been described previously (Rodrigues & Kunavongkrit,

1983). The catheter was brought through a subcutaneous tunnel to the back between the scapulas. After being exteriorised, the catheter was put in a pocket made of canvas on the dorsal aspect of the pig neck. The pocket was fixed at several points with steel sutures. The catheter was cut off to a suitable length and was flushed twice a day with saline and heparin solution (5000 IU heparin in 0.9% NaCl). The antibiotic, procaine benzylpenicillin (Etacilin®; Intervet AB, Stockhom, Sweden) was administered at 20 mg/kg i.m. on the day of surgery and the two following days. The regular feeding regime was restored immediately after recovery from anaesthesia. After insertion of the jugular catheter, the pigs were kept in individual pens within sight and sound of one other. Eight days later, a third exercise test was performed (using the same exercise protocol as the second exercise test) and tissue samples from the left *m. biceps femoris* were taken within 10 minutes of completion of exercise (Papers II, IV) and from the right *m. biceps femoris* after 6 hours of recovery (Papers II, IV). In addition, blood samples were taken from the indwelling jugular catheter before exercise, immediately after the pigs stopped exercising and then after 10 min, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h and 6 h of recovery (Paper II).

The pigs were then 18 to 20 weeks old and the carriers had a mean weight of 80 ± 1.5 kg and the non-carriers a mean weight 74 ± 3 kg, with no significant difference between the two genotypes.

One week later, tissue samples were taken from *m. biceps femoris*, *m. longissimus dorsi*, *m. masseter* and *m. semitendinosus* by excision, after the animals had been euthanised by an intravenous overdose of pentobarbital (100 mg/mL) (Paper III). Two pigs were withdrawn from the study after training, one due to unwillingness to run on the treadmill and the other did not survive anaesthesia.

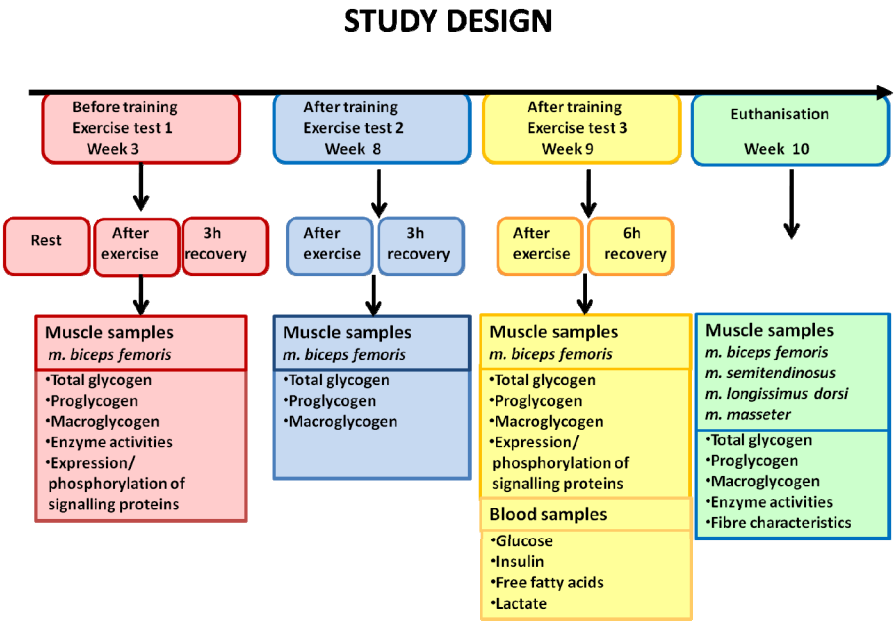

Figure 5. An overview of muscle samples and blood samples obtained in the thesis.

3.3 Muscle samples

Muscle biopsy specimens (about 2x1x1 cm) were obtained from *m. biceps femoris* at a depth of 3-4 cm. Muscle specimens were also obtained from *m. biceps femoris*, *m. masseter*, *m. semitendinosus* (white portion) and *m. longissimus dorsi* (caudal to the last rib) from the centre of the middle part of the muscle within 10 minutes of death. The tissue samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis. The tissue samples used for histochemistry were rolled in talcum powder before being frozen.

3.3.1 Western Blot (Papers I, II)

Muscle biopsies were freeze-dried overnight and then muscle tissue was dissected out under a microscope to remove visible blood, fat and connective tissue. Samples were subsequently homogenised in ice-cold homogenisation buffer containing 20 mM Tris (pH 7.8), 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl2, 1% Triton X-100, 10% (w/v) glycerol, 10 mM NaF, 1 mM ethylenediaminetetraacetic acid (EDTA), 5 mM Na-pyrophosphate, 0.5 mM Na3V04, 1 µg/mL leupeptin, 0.2 mM phenylmethyl sulphonyl fluoride, 1 µg/mL aprotinin, 1 mM dithiothreitol

(DTT), 1 mM benzamidine and 1 μM microcystin. The homogenised samples were then rotated for 30 min at 4° C and thereafter subjected to centrifugation (12 000g for 15 min at 4°C). Protein concentration in the supernatant was determined using BCA protein assay kit (Pierce, Rockford, IL, USA). Phosphorylation of protein kinases was determined by immunoblot analysis. Skeletal muscle protein lysate (40 μg) was separated by SDS-PAGE (7.5% resolving gel), transferred to PVDF membranes and blocked with 7.5% non-fat milk in TBS-T for two hours at room temperature. Membranes were incubated with primary antibodies: anti-pGSK3 α/β $Ser^{21/9}$, pAS160, AS160, Akt, pAkt Thr^{308}, pAkt Ser^{473}, panAMPKα and pAMPK Thr^{172} (Cell Signaling Technology; Boston, MA), GLUT4, $Na^{+}K^{+}$-ATPase α2-subunit and pacetyl-CoA carboxylase (pACC) Ser^{79}(Upstate/ Millipore Corporate Headquarters; Billerica, MA), glycogen synthase (gift from Prof. Erik Richter, Copenhagen University), overnight at 4°C on a shaking platform. Thereafter, membranes were washed with TBS-T and incubated with appropriate anti-rabbit or anti-mouse secondary antibody. Samples from pigs with or without the *PRKAG3* mutation were included and analysed on the same gel in parallel. Proteins were visualised by enhanced chemiluminescence (ECL, Amersham, Little Chalfont, Buckinghamshire, UK) and quantified by densitometry and molecular analyst software (Bio-Rad, Richmond, CA, USA). All membranes were normalised for loading with glyceraldehyde-3-phophate dehydrogenase (GAPDH; Santa Cruz Biotechnology, Santa Cruz, CA, USA).

3.3.2 Enzyme activity (Papers I, III)

Muscle biopsies were freeze-dried overnight and then muscle tissue was dissected out under a microscope to remove visible blood, fat, and connective tissue. To determine the activity of citrate synthase (CS), 3-hydroxyacyl-CoA dehydrogenase (HAD), lactate dehydrogenase (LDH), hexokinase (HK), and phosphorylase, 1-2 mg of pure tissue was homogenized with an ultrasound disintegrator (Branson) in ice-chilled potassium phosphate buffer (0.1 M, pH 7.3) at a dilution of 1:400 and then analysed fluorometrically (Lowry & Passoneau, 1973). Glycogen synthase (GS) activity was determined by a modified method by Thomas *et al.*, (1968), based on incorporation of 14C UDPG into glycogen. The active form of GS activity was measured at a low concentration (0.3mM) and the total activity at a high (6.0mM) concentration of glucose-6-phosphate.

3.3.3 Glycogen (Papers I-IV)

For glycogen determination, 1-2 mg of pure tissue was boiled in 1 M HCl for 2 h to form glucose residues. Glucose was analysed by a fluorometric method (Lowry & Passoneau, 1973).

3.3.4 Proglycogen and macroglycogen (Paper IV)

Fractions of proglycogen and macroglycogen were separated on the basis of solubility in PCA, using the method described by Adamo & Graham (1998). In brief, 200 μL of ice-cold 1.5M PCA were added to 1.5 mg of freeze-dried muscle specimens. The extraction of macroglycogen into the PCA continued for 20 min on ice. After centrifugation at 4000 rpm and 4°C for 10 min, 100 μl of the supernatant was removed and used for analysis of macroglycogen. The remaining supernatant was used for analyses of glucose, glucose-6-phosphate and lactate and the pellet was used for analysis of proglycogen content. Glycogen was hydrolysed by the addition of 1mL of 1 M HCl to the proglycogen and macroglycogen fraction. The sample tubes were sealed and heated to 100°C in a water bath for 2 hours, after which they were transferred to Eppendorf tubes and stored at -80°C until fluorometrically analysed for glycosyl units (Lowry & Passoneau, 1973).

3.3.5 Glucose, glucose-6-phosphate and lactate (paper IV)

Glucose and glucose-6-phosphate were analysed fluorometrically (Lowry & Passoneau, 1973) and lactate was analysed using a lactate kit (EnzyPlus, Biocontrol System, Denmark).

3.3.6 Muscle fibre characteristics (Paper III)

The muscle sample was mounted on embedding medium (OCT compound) and serial transverse sections (10 μm) were cut in a cryostat (2800 Frigocut E, Reichert-Jung, Leica Microsystems GmbH) at -20°C. Myofibrillar ATPase staining with pre-incubation at pH 4.3, 4.6 and 10.3 was used to identify fibre types I, IIA, IIB (Brooke & Kaiser, 1970) in all muscles. In *m. biceps femoris* and *m. longissimus dorsi* immunohistochemical methods were also used. Serial sections were reacted with myosin heavy chain (MHC) antibodies BA-D5 (MHCI) (gift from E.Barrey) and A4-74 (MHCIIA + MHCIIX) (Alexis Biochemicals). The secondary antibody (rabbit anti-mouse immunoglobulins) and the peroxidase-anti-peroxidase complex used to visualise the binding to the antibody were obtained from

Dako in Denmark. Sections (containing at least 200 fibres) of the pH 4.6 ATPase stain were photographed and type IIB fibres on this section corresponding to fibres that stained with the A4-74 antibody were classified as type IIAX fibres. All type I fibres from the ATPase stain corresponded to type I fibres stained with the antibody BA-D5 (MHCI). The muscle fibres stained with the antibody A4-74 were classified as IIAX fibres and some of these may be pure IIX and/or IIBX fibres. A computerised image analyser (Bio-Rad, Scan Beam, Hadsund, Denmark) was used to evaluate fibre type composition, fibre type area and relative fibre type area. Sections of *m. biceps femoris* and *m. longissimus dorsi* were also stained with the NADH tetrazolium reductase method (Novikoff *et al.*, 1961). Oxidative capacity was subjectively evaluated from the intensity of the blue staining (30-50 fibres of each type) as high (the whole fibre stained), medium (some staining apparent, mostly at the cell borders), or low (hardly any staining within the cell). A semi quantitative estimate of glycogen content in muscle fibres was obtained from sections stained with periodic acid-Schiff (PAS).

3.4 Blood samples (Paper II)

The blood samples were immediately put on ice until they were centrifuged to obtain plasma or serum, which was stored at - 80° C until analysis. Plasma lactate concentrations were analysed using the Analox Lactate Analyser (Analox Instruments LTD, London W6 0BA, UK). Free fatty acid (FFA) concentrations in plasma were analysed using a FFA kit from Wako (NEFA C test, Wako Chemicals, GmbH, Neuss, Germany) Glucose concentrations in plasma were analysed using a fluorometric technique (Lowry & Passonneau, 1973). Insulin concentrations in serum were analysed by a radioimmunoassay technique, Pharmacia Insulin RIA 100 and Coat-A-Count (DPC Los Angeles, USA) at the clinical chemical laboratory.

Figure 6. Photos showing pigs trotting on the treadmill, blood sampling from a jugular catheter, and a shower after exercise.

3.5 Statistical analyses

In Papers I, II and IV, a rank sum test was used for comparison of values from the carrier and non-carrier pigs. For comparison of the consecutive biopsy specimens within carriers or non-carriers, a signed rank test was used. In Paper III an unpaired t-test was used for comparison of values from the carrier and non-carrier pigs. The level of statistical significance was set at $P < 0.05$. Statistical analyses were carried out using Sigma Stat Statistical Software version 11.0 (Aspire Software International, 20448 Charter Oak Drive, Ashburn, VA, 20147, USA).

In Paper II the following statistical model was used for the blood data pre- and post-exercise.

$yijt = \mu + ai + pij + bt + (ab)jt + eijt$

where μ=overall expectation, ai=(fixed) effect of group i, pij=(random) effect of pig j within group i, bt=(fixed) effect of time t, (ab)jt =(fixed) interaction effect of group and time, and eijt = (random) residual error. Heteroscedastic variation was assumed for the errors with different variances for the time levels. Differences between groups were tested with the pig variation included, whereas the time and interaction effects were tested considering the error variation only. Proc Mixed of the SAS statistical package was used for the numerical evaluations.

4 Main results and discussion

4.1 Glycogen, Proglycogen and Macroglycogen (Papers I-IV)

4.1.1 Exercise tests

The carriers of the *PRKAG3* mutation had, as expected, higher muscle glycogen storage mainly in the macroglycogen fraction compared with the non-carriers before exercise, immediately after exercise and 3 h into the recovery phase. Furthermore, the carriers had higher macroglycogen content immediately after exercise and 3 h and 6 h into the recovery phase after a training period. A similar degradation of glycogen after the exercise test before and after the training period took place in both carriers and non-carriers. A re-synthesis of macroglycogen occurred after 3 h of recovery both before and after the training period in the carriers, whereas this was not observed in the non-carriers. After 6 h of recovery this difference was no longer seen between the genotypes.

The results from the exercise tests before and after a training period confirm the faster re-synthesis of glycogen observed in carriers after exercise in a previous study on a few pigs (Essén-Gustavsson *et al.*, 2005). It has also been reported that glycogen re-synthesis 2.5 hours after swimming is accelerated in transgenic mice (Tg-Prkag3^{225Q}) expressing the natural pig mutation (Barnes *et al.*, 2004). The study on mice suggested that the mutation caused a greater reliance on lipid oxidation and that triglyceride content in muscle decreased in response to exercise. A study in humans with the γ3 R225W mutation also provides evidence to suggest that the *PRKAG3* mutation influences not only glycogen metabolism but also lipid metabolism. The γ3 R225W mutation in humans causes a 90% increase in skeletal glycogen content and a 30% decrease in intramuscular triglyceride stores (Costford *et al.*, 2007). Increased lipid oxidation may reduce the

demand for glucose oxidation and thus more glucose within the muscle can be directed toward glycogen synthesis.

Several studies have shown that when the total glycogen concentration in the muscle reaches a concentration over 300-350 mmol glucosyl units/kg dw, the re-synthesis of glycogen occurs mainly in the macroglycogen pool in man (Jansson *et al.*, 1981; Adamo *et al.,* 1998). A similar process occurs in the carrier pigs, since they showed a higher re-synthesis of macroglycogen in early recovery, whereas this was not observed in the non-carriers. As can be seen from the results, the re-synthesis of glycogen after 6 h of recovery occurred in the proglycogen fraction in both genotypes. In the carrier pigs, macroglycogen re-synthesis preceded proglycogen re-synthesis in the early recovery phase, possibly due to the higher initial concentration of glycogen.

4.1.2 After euthanasia

The carriers of the *PRKAG3* mutation had higher total glycogen content in *longissimus dorsi*, *m. semitendinosus*, *m. biceps femoris* and *m. masseter* compared with the non-carriers. It was the macroglycogen fraction that was higher in the muscles. A previous study has shown that the mutation mainly affects muscle characteristics of white glycolytic muscles such as *m. longissimus dorsi* and has no effect on red muscles such as *m. semispinalis capitis* (Lebret *et al.*, 1999). This is presumably related to the fact that the γ3 isoform of AMPK is the predominantly expressed form, especially in muscles containing type IIB fibres (Mahlapuu *et al.*, 2004). In agreement with earlier studies on untrained pigs, the pigs carrying the *PRKAG3* mutation had a higher content of glycogen in *both m. longissimus dorsi* and *m. biceps femoris* than the non-carriers (Enfält *et al.*, 1997; Essén-Gustavsson *et al.*, 2005). Although *m. masseter* is considered to be a red muscle based on high CS activity and low glycolytic potential (Monin *et al.*, 1992; Karlström, 1995), the carriers of the *PRKAG3* mutation had higher macroglycogen content than the non-carriers. According to the fibre characteristics of the muscle, some glycolytic type II fibres exist in *m. masseter* (Paper III) and they are probably influenced by the mutation, resulting in overall higher glycogen content.

The total glycogen values of the muscles were high after euthanasia, but the values are difficult to compare with other studies, since the pigs had been training for 5 weeks and training has been shown to increase the glycogen content in certain muscles (Essén-Gustavsson *et al.*, 1988). Furthermore, in order to avoid a stressful situation, the pigs were euthanised with an overdose of pentobarbital through a jugular catheter in their home pen by a well-known person and thereafter sampled. In previous studies, the

glycogen content in the carriers has mostly been determined by measuring the glycolytic potential. Glycolytic potential estimates the glycogen present at slaughter and includes glycogen, as well as lactate, glucose and G-6-P, i.e. products and intermediates of *post mortem* glycolysis (Monin & Sellier, 1985). Pre-slaughter handling of the animals affects the glycolytic potential. Both glucose and glycogen content are known to be easily affected by stress, which means that whether animals were transported and how they are killed may have an important impact upon the glycogen levels. For example, it has been shown that 0-60% of IIB fibres are depleted after slaughter in an abattoir (Karlsson, 1993). This can be compared with the fact that there was no depletion pattern after death in the carrier and non-carrier pigs in the present study.

It has been shown that the carriers of the *PRKAG3* mutation have hyper-accumulation of glycogen and an abnormally enlarged sarcoplasmic compartment, mainly in glycolytic muscle fibres (Estrade *et al.* 1993). Fibres that have a high oxidative capacity, such as type I and type IIA fibres, may be less affected by the mutation and since carrier pigs have a higher macroglycogen content, it is likely that IIB fibres contain more macroglycogen.

According to studies separating glycogen by electrophoresis and by transmission electron microscopy (Skurat *et al.*, 1997; Marchand *et al.*, 2002), the proglycogen and macroglycogen may not represent two distinct fractions, but merely subfractions of a continuous distribution of sizes of glycogen particles. It is important to bear in mind that the glycogen content in the present study was measured in a sample that represented the whole muscle, whereas previous studies have shown that glycogen is not consistently distributed within the cell (Fridén *et al.*, 1989; Marchand *et al.*, 2002). In human skeletal muscle, the type and duration of exercise have been shown to have implications for where glycogen degradation occurs within the muscle fibre (Fridén *et al.*, 1989). For example, the sarcolemmal glycogen fraction in type II fibres is depleted during short-term high intensity exercise, whereas it remains largely unaffected in type I fibres during long-term aerobic exercise. Aerobic exercise primarily results in degradation of glycogen from the intramyofibrillar regions within the muscle fibres (Fridén *et al.*, 1985; Fridén *et al.*, 1989). Different subcellular location of proglycogen and macroglycogen within the muscle cell could result in different susceptibility to enzymes such as glycogen synthase and glycogen phosphorylase. The degree of activation of AMPK is shown to be inversely related to glycogen concentration (Richter *et al.*, 2001; Hargreaves, 2004) and it has also been shown that AMPK may be able to

sense the status of the cellular energy reserves in the form of glycogen, as it has a glycogen-binding domain (McBride *et al.*, 2009). One may speculate that the structure and localisation of the glycosomes may differ between genotypes and that this is a factor to consider for the degree of AMPK activation in association with exercise.

4.1.3 Exercise performance

Studies of humans with a spontaneous *PRKAG3* mutation and transgenic mice expressing the *PRKAG3* mutation have indicated that the mutation results in improved performance in mice and humans (Barnes *et al.*, 2005; Crawford *et al.*, 2010). Investigating this phenomenon in trained pigs with the natural mutation did not result in the same conclusion. Instead, the results were more in agreement with a previous study on a few untrained carrier pigs (Essén-Gustavsson *et al.*, 2005). There were great individual differences both between and among carriers and non-carrier pigs in terms of time until exhaustion and the carriers did not perform better than the non-carriers. By thoroughly scrutinising human and transgenic mouse studies of the *PRKAG3* mutation, it was found that the training protocols in the different studies were not comparable to the distance test performed in present study. The human study was more of a strength training (leg extensions of quadriceps muscles) than endurance training character. Furthermore, the study with transgenic mice (*Tg-Prkag3*^{225Q}) was conducted by repeated electrical stimulation of an isolated muscle, *m. extensor digitorum longus*. This is quite different from treadmill running *in vivo*, especially since rodent muscles are usually composed of only one fibre type (in this case white fast-twitch), which is easy to stimulate electrically.

4.2 Effect on insulin and/or contraction induced signalling pathways (Papers I, II)

4.2.1 Before exercise training

It was demonstrated that increased glycogen synthesis in pigs carrying the *PRKAG3* mutation is related to an increased exercise-mediated Akt Ser^{473} dependent signalling response in skeletal muscle and increased phosphorylation of AS160 compared with non-carriers. The enhanced phosphorylation of Akt and AS160 observed in carriers of the *PRKAG3* mutation after treadmill exercise supports exercise-stimulated signalling, which may result in an increased glucose influx into the skeletal muscle

cells. Phosphorylation of GSK3α or GSK3β did not differ between the two genotypes, nor did the expression of GS or the percentage of active to total form of GS activity. Previous studies have shown that phosphorylation of Akt activates GSK3, which then influences GS activity and glycogen synthesis (Cross *et al.*, 1995). In pigs, this signalling system did not seem to be affected by the mutation or acute exercise on a treadmill. The GS enzyme activity at rest has been investigated in *m. longissimus dorsi* of pigs carrying the *PRKAG3* mutation by Estrade *et al.* (1994). The study found no significant differences between the two genotypes, but showed that the branching enzyme activity was twice as high in the muscles, whereas no differences were seen regarding the phosphorylase or debranching enzyme activity.

To determine the functional role of the AMPK γ3 isoform, *Tg-Prkag3* ^{225Q} mice have been created. The mice show elevated skeletal muscle glycogen content, normal glycogenolysis during exercise and accelerated glycogenesis after exercise, increased intramyocellular triglyceride utilisation during exercise, increased phosphorylation of acetyl-CoA carboxylase (ACC) immediately after exercise but not in recovery, and a reduction in phosphorylation of glycogen synthase during recovery. In the *Tg-Prkag3*^{225Q} mice, the mutation seems to protect against diet induced insulin resistance by attenuating intramyocellular triglyceride accumulation (Barnes *et al.*, 2004).

A study on humans with the γ3 R225W mutation also provides evidence that the *PRKAG3* mutation (R225W) influences not only glycogen metabolism but also lipid metabolism. The γ3 R225W mutation in humans leads to a 90 % increase in skeletal glycogen content and a 30 % decrease in intramuscular triglyceride stores (Costford *et al.*, 2007). Increased lipid oxidation may reduce the need for glucose oxidation and thus more glucose within the muscle can be directed toward glycogen synthesis.

The *PRKAG3* mutation in pigs has previously been suggested to be a gain-of-function mutation, leading to increased glucose transport and glycogen synthesis (Milan *et al.*, 2000). It has been shown that AMPK activity in resting muscle extracts of pigs is about three-fold lower in carriers than in non-carriers, both in the presence and absence of AMP (Milan *et al.*, 2000). Interestingly in the present study there was a tendency for the carriers to have a lower expression of AMPK, which may indicate that the *PRKAG3* mutation has an influence on AMPK.

4.2.2 After exercise training

Carriers of the *PRKAG3* mutation which were exercise-trained for five weeks had lowered expression of AMPK and increased phosphorylation of Akt Ser^{473} after exercise compared with non-carriers. Acute exercise stimulated the phosphorylation of AS160 in both genotypes, and the phosphorylation of GSK3αSer^{21} and ACC Ser79 in the non-carriers. An increase in pAkt Ser^{473} due to exercise is in agreement with exercise studies on humans (Sakamoto *et al.*, 2004) and rats (Sakamoto *et al.*, 2003) and was also observed in untrained carriers (Paper I). A point of interest is that when pAkt Ser^{473} values after exercise were high in trained carriers of the *PRKAG3* mutation, the muscle glycogen storage was also high. This has also been shown in a study on humans, in which pAkt Ser^{473} was elevated more post resistance exercise when subjects had been on a high-carbohydrate diet causing high muscle glycogen levels in comparison with a low carbohydrate diet causing low muscle glycogen levels (Creer *et al.*, 2005). The trained non-carriers in the present study only had a tendency for higher pAkt Ser^{473} values after exercise and they had lower muscle glycogen levels. The increased signalling response of Akt after exercise observed in the trained carriers and the increase in pAS160 may be related to an increased glucose influx directed towards glycogen synthesis in the recovery period. However, the differences seen between genotypes in pAMPK Thr^{172} and AMPK expression indicate that altered AMPK activation may play a role for the enhanced glycogen storage in the carriers. The lowered AMPK activity which has been shown in carrier pigs is likely to reflect feedback inhibition due to the high energy status in the muscle (Milan *et al.*, 2000). The lower expression of AMPK and pAMPK Thr^{172} in the trained carriers may thus be related to feedback inhibition by the enhanced glycogen stores. When there is a high energy demand in the muscle, AMPK is usually activated in order to stimulate glucose uptake and fatty acid oxidation and inhibit glycogen synthesis. This was likely to have occurred during the exercise bouts in the training period. However, if large glycogen stores are associated with AMPK inhibition, glycogen synthesis may be stimulated and not inhibited. Indeed, it has been shown that AMPK may sense the status of the cellular energy reserves in the form of glycogen, as it has a glycogen binding domain (McBride *et al.*, 2009). The increase in pACC Ser^{79} and the pAMPK Thr^{172}/AMPK ratio after exercise in the non-carriers in the present study indicates that AMPK was more activated in these pigs. These results from the trained non-carriers are in good agreement with findings in human exercise studies reporting increases in pAMPK Thr^{172} and pACC Ser^{79} concurrent with reductions in muscle glycogen content (Stephens *et al.*,

2002; Chen *et al.*, 2007). Inhibition of ACC by pAMPK Thr^{172} is suggested to be an important component in stimulating fatty acid oxidation in response to exercise.

A noteworthy finding was that after 6 h of recovery from exercise the re-synthesis of glycogen did not differ between the genotypes, although the glycogen levels at this time differed markedly. The altered signalling response after exercise in the pigs carrying the *PRKAG3* mutation thus seems to influence only the initial re-synthesis rate of glycogen.

4.3 Metabolic blood responses (Paper II)

There was no marked effect on insulin and blood glucose concentrations in response to exercise in the trained carriers of the *PRKAG3* mutation that could be associated with their high capacity to store glycogen. These results indicate that glucose uptake and the high glycogen storage capacity in muscles of carriers of the *PRKAG3* mutation are not related to a different insulin response. This is also supported by the results of intravenous glucose tolerance tests that were performed in both carriers and non-carriers of the *PRKAG3* mutation, which showed no difference between the genotypes, whether trained or untrained (Granlund *et al.*, unpublished observations). The similar lactate response to exercise in both carriers and non-carriers of the mutation indicates a similar degree of anaerobic metabolism even if initial glycogen levels differed between genotypes. These results are in good agreement with the lactate response observed after exercise of untrained carriers and non-carriers of the *PRKAG3* mutation (Essen-Gustavsson *et al.*, 2005). Whether fatty acid oxidation differs between genotypes in response to exercise or in the recovery period was not evaluated here. There was no limitation in availability of fatty acids in blood in the early recovery period. Intramuscular triglyceride stores are another source that could release fatty acids. Notably, humans with the γ3R225W mutation have increased glycogen stores and decreased triglyceride stores (Costford *et al.*, 2007). Glycogen synthesis in muscle following exercise could be a result of increased fatty acid oxidation leading to a glucose sparing effect that promotes glycogen synthesis. Other factors that could influence the re-synthesis of glycogen after exercise are differences in metabolic properties among fibre types, localisation of enzymes and glycogen storage within the fibres.

4.4 Muscle characteristics (Papers I, III)

4.4.1 Enzyme activity

Before exercise training

At rest, the enzyme activity of HK and phosphorylase was higher in carriers but no differences concerning CS, HAD, LDH and GS were observed in *m. biceps femoris*. The activity of HK and phosphorylase was higher in the carriers than the non-carriers after exercise and 3 h into the recovery phase.

In earlier studies, CS and HAD activity has been shown to be increased and LDH decreased in a white muscle, *m. longissimus dorsi*, of pigs carrying the *PRKAG3* mutation (Estrade *et al.*, 1994; Lebret *et al.*, 1999). In a red muscle, *m. semispinalis capitis*, the mutation has no influence on enzyme activity (Lebret *et al.*, 1999). An earlier study on carriers and non-carriers of the *PRKAG3* mutation has shown that *m. biceps femoris* contain 23% type I and 77% type II fibres with no differences seen between genotypes (Essén-Gustavsson *et al.*, 2005). However, the aforementioned study of *m. longissimus dorsi* showed that the latter muscle is composed of 10% type I and 90% type II fibres, with no differences between genotypes regarding fibre type. Type II fibres primarily express the AMP-kinase γ3 (Mahlapuu *et al.*, 2004), and thus metabolism in muscles composed of many type II fibres may be influenced to a greater extent by the mutation. Interestingly, HK activity was significantly higher in the *m. biceps femoris* of the carriers in the present study, as was phosphorylase activity, indicating that these enzymes are influenced by the mutation.

After exercise training after euthanasia

The CS activity was higher in the carriers of the *PRKAG3* mutation than in the non-carriers only in *m. longissimus dorsi,* and there was no difference between genotypes regarding HAD activity in *m. longissimus dorsi*, *m. semitendinosus* or *m. biceps femoris*. The activity of LDH in *m. longissimus dorsi* and *m. semitendinosus* was lower in the carriers of the *PRKAG3* mutation than in the non-carriers. The HK activity was higher in all muscles in the carriers and the activity of phosphorylase was higher in *m. biceps femoris* and *m. longissimus dorsi* in the carriers compared with the non-carriers.

A noteworthy finding was that the HK activity was significantly higher in the carrier pigs in all three muscles sampled, indicating a higher capacity for phosphorylation of glucose in the glycogen synthesis pathway. This result had already been observed regarding *m. biceps femoris* in non-trained carriers (Paper I). Further evidence that this pathway is affected by the mutation is

supplied by the fact that expression and activity of the UDP-glucose pyrophosphorylase of *m. longissimus dorsi* are up-regulated in pigs carrying the *PRKAG3* mutation (Hedegaard *et al.*, 2004), and that carriers of the mutation also have higher branching enzyme activity in *m. longissimus dorsi* (Estrade *et al.*, 1994). These data on enzyme activity support that high muscle glycogen content due to the *PRKAG3* mutation is due to an increased synthesis of glycogen.

The *PRKAG3* mutation may also have an effect on glycogenolysis in association with high muscle glycogen storage as indicated by the higher phosphorylase activity found in both *m. longissimus dorsi* and *m. biceps femoris* in the carriers. The higher phosphorylase and HK activity observed in *m. biceps femoris* of the exercise-trained carriers is in agreement with results on untrained carriers (Paper I). This indicates that the *PRKAG3* mutation has a great influence on these enzymes and may suggest that carriers of the mutation have increased glycogen turnover. In a previous study the HAD activity was higher in *m. longissimus dorsi* (Lebret *et al.*, 1999), but this was not seen in any of the muscles in the present study. The CS activity was higher in the carriers of the *PRKAG3* mutation than in the non-carriers only in *m. longissimus dorsi*, which is in accordance with the study on untrained carriers (Lebret *et al.*, 1999). The decreased glycolytic capacity in *m. longissimus dorsi* and *m. semitendinosus* of the carriers is also in accordance with the study on untrained pigs (Lebret *et al.*, 1999).

4.4.2 Fibre characteristics

No differences between genotypes were seen when fibre type composition was evaluated with the myosin ATPase method. However, immunohistochemical methods showed that compared with the non-carriers, the carriers had a higher percentage of type II fibres stained with the antibody identifying type IIA and IIX fibres in *m. longissimus dorsi* and a lower percentage of type IIB fibres in both *m. biceps femoris* and *m. longissimus dorsi*. In these muscles the relative area of type IIB fibres was lower in carriers than in non-carriers.

Previous studies of fibre characteristics in *m. longissimus dorsi* in pigs carrying the *PRKAG3* mutation indicate that alterations may occur in the subgroups of type II fibres (Lebret *et al.*, 1999; Park *et al.*, 2009), which is also in agreement with the findings of the present study. A notable feature was that the carriers of the *PRKAG3* mutation had less IIB fibres, not only in *m. longissimus dorsi*, but also in *m. biceps femoris*, compared with the non-carriers. It has previously been indicated that adaptations to training may

differ between these muscles (Essén-Gustavsson *et al.*, 1980; Essén-Gustavsson *et al.*, 1988). Endurance-trained pigs had an increased oxidative capacity and a higher glycogen content in *m. biceps femoris* compared with non-trained pigs, but no differences were seen in *m. longissimus dorsi* and *m. semitendinosus*, muscles considered to be less involved during training on a treadmill (Essén-Gustavsson *et al.*, 1980; Essén-Gustavsson *et al.*, 1988). The oxidative capacity evaluated by CS activity in the present study for the different genotypes did not differ in *m. biceps femoris* but differed in *m. longissimus dorsi*, which may be related to the different involvement of these muscles during locomotion. The results for *m. longissimus dorsi* were similar to earlier results on untrained pigs and indicate that this muscle is not as much involved during treadmill exercise and therefore does not have to adapt to training. In both genotypes, training adaptations in the fibres of *m. biceps femoris* may have caused a similar oxidative capacity in response to the increased energy demand during locomotion. PAS staining showed that glycogen was lowered in both genotypes in type I, IIA and some IIB fibres in *m. biceps femoris* after exercise (Paper IV), which indicates that these fibres had been recruited. Adaptations to exercise training in this muscle may have decreased the effects of the *PRKAG3* mutation on muscle metabolic and contractile properties. A study with transgenic mice models showed that mice with a chronically AMPK-activating mutation caused a shift from fibre type IIB to IIA/X fibres, had higher activity of CS and increased hexokinase protein expression, regardless of whether these mice had exercised or not (Röckl *et al.*, 2007). AMPK signalling was suggested to play an important role for transforming skeletal muscle fibre types as well as for increasing hexokinase II protein expression and oxidative capacity. These findings are in agreement with effects of the *PRKAG3* mutation on muscle characteristics presented in this thesis, especially in *m. longissimus dorsi*.

The carriers had less type IIB fibres in *m. longissimus dorsi* which indicates that one effect of the *PRKAG3* mutation may be associated with transformation of type IIB towards type IIX and IIA fibres, as carriers also had more type IIAX fibres. The muscle fibres that are classified as MHCIIAX may be a mixture of pure IIX and/or hybrid IIA+IIX and IIX+IIB as the antibody A4-74 identifies both IIA and IIX fibres (Graziotti *et al.*, 2001; Fazarinc *et al.*, 2009). Studies in pigs indicate that fibre type shifts from type IIB to IIA may occur with training (Essén-Gustavsson *et al.*, 1993; Pedersen *et al.*, 1998). Oxidative capacity is known to increase with training and among fibre types oxidative metabolism is high in type I fibres and decreases in the order type I > type IIA > type IIX > type IIB fibres (Lefaucheur *et al.*, 2002). Modern pig production has resulted in intensive

selection for increasing muscle yield and lean muscle growth, which has not only caused fibre type transformation, but also induced an alteration in muscle metabolism towards a more glycolytic and less oxidative fibre type (Lefaucher, 2010). In contrast, the *PRKAG3* mutation has been shown to decrease IIB and increase IIA and IIX mRNA expression (Park *et al.*, 2009), which also implies that the genotype promotes a more oxidative phenotype. The changes seen in muscle characteristics in carriers of the *PRKAG3* mutation thus resemble those seen when muscles in pigs adapt to an increased level of physical activity, independent of contractile activity. This is supported by the higher CS activity and the higher oxidative capacity of type IIB muscle fibre types according to the NADH-tetrazolium reductase staining found in *m. longissimus dorsi* of the carriers. Studies have shown that even large animals such as pigs and llamas express MHCIIB fibres, mostly in glycolytic muscles (Lefaucheur *et al.*, 1998; Graziotti *et al.*, 2001). The non-carriers in the present study showed 47% type IIB fibres in the *m. longissimus dorsi*, which is in good agreement with a previous study in pigs (Lefaucheur *et al.*, 2002)

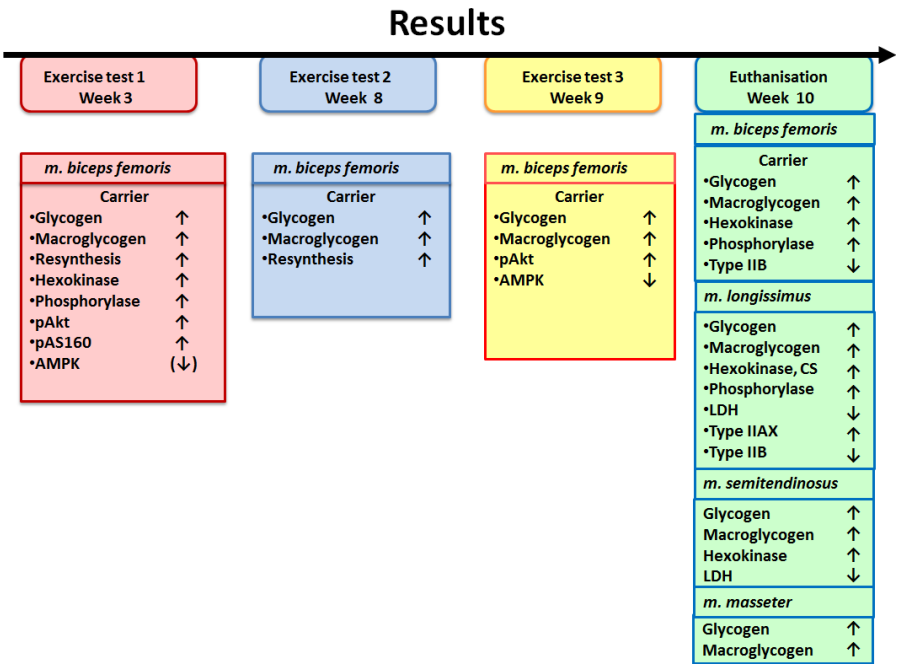

Figure 7. Summary of main results of the thesis.

5 Conclusions

- Expression of AMPK in muscle was lower in pigs carrying the *PRKAG3* mutation than in non-carriers. The increased rate of glycogen synthesis following exercise in carriers was correlated with an increased signalling response of Akt and its substrate AS160, and a higher activity of hexokinase, indicating an increased glucose influx and phosphorylation of glucose, directed towards glycogen synthesis

- Alterations in the blood concentrations of insulin, glucose, lactate and free fatty acids before and after an exercise test and in the recovery phase did not differ between carriers and non-carriers of the *PRKAG3* mutation.

- Immunohistochemical methods showed that carriers of the *PRKAG3* mutation had a lower percentage of type IIB fibres in *m. biceps femoris* than non-carriers. In *m. longissimus dorsi* there was a lower percentage of type IIB fibres and a higher percentage of type IIAX fibres. In both these muscles the relative area of type IIB fibres was lower in carriers than non-carriers. The *PRKAG3* mutation influences muscle characteristics in exercise-trained pigs and promotes a varying degree of oxidative phenotype among muscles with different functions.

- Carriers of the *PRKAG3* mutation had higher total glycogen content in all sampled skeletal muscles than non-carriers, with the macroglycogen fraction being higher. At 3 h but not at 6 h of recovery following exercise, re-synthesis of glycogen, especially of macroglycogen, was higher in carriers than in non-carriers.

6 Aspects of future work

This thesis examined some factors behind the high capacity of pigs with *PRKAG3* mutation to store and re-synthesise glycogen. Further studies are needed to continue this investigation.

- In this thesis, studies have been performed on samples that represented the whole muscle. A step towards further knowledge would be to determine the degradation and re-synthesis of glycogen and its fractions (proglycogen and macroglycogen) within a fibre and between different fibre types. This could be achieved by using for example a confocal microscope to study subcellular structures and their relation to glycogen within the muscle cell. Different subcellular locations of proglycogen and macroglycogen within the muscle cell could result in different susceptibility to enzymes.
- The carriers have in this study, showed lower expression of AMPK. A step further would be to measure the activity of AMPK and also combining immunohistochemistry with ordinary histochemistry, to determine if there are differences in the expression of AMPK or other signalling proteins such as Akt between fibre types. In this study the antibody against AMPK α-subunit has been used. Today the antibody against AMPK-γ subunit is available and if it works in pigs, the antibody could be of interest to use.
- Combining conventional histochemistry with immunohistochemistry will result in a better understanding of the muscle and how it adjusts to different stimuli. In particular antibodies that can separate MHCIIA and MHCIIX fibres in carrier and non-carrier pigs are of interest. This study clearly shows that using fibre typing according to the MHC isoforms results in extended knowledge.

- A more intense exercise protocol with intervals of trotting to recruit more type IIB fibres. An exercise test after 3 h of recovery could also be of interest, since the carriers re-synthesise more glycogen at this time point.
- Fat is another important energy source. There are studies in humans with the γ3 R225W mutation and transgene mice (Tg-Prkag3^{225Q}) which indicate that the *PRKAG3* mutation could be associated with increased lipid oxidation and in the presence of increased lipid supply attenuate the expected accumulation of intramuscular triglyceride content. It would therefore be of interest to investigate the intramuscular triglyceride content in carriers of the *PRKAG3* mutation and also in relation to different diets.

7 Populärvetenskaplig sammanfattning

Ett flertal grisar av Hampshire ursprung har den dominanta RN mutationen vilket medför att dessa grisar har ett nästan fördubblat glykogeninnehåll i sin muskulatur. Mekanismen bakom det höga glykogeninnehållet är inte känt men mutationen har visat sig vara ett enkelt basparsbyte i PRKAG3 genen. Mutationen leder till en förändring i enzymet AMP kinas som medverkar i regleringen av glukosomsättningen vid metabolisk stress. Glukos transporteras in i cellen med hjälp av bl.a. insulin och lagras till stor del som glykogen i skelettmuskulaturen. Glukosmolekylen kan inte diffundera fritt in i cellen utan behöver hjälp av transportproteiner. Muskel och fettväv uttrycker en speciell glukostransportör, GLUT4, som i basalt tillstånd återfinns inuti själva cellen. Med hjälp av speciella signaleringssystem förflyttas GLUT4 till plasmamembranet och gör det möjligt för glukos att passera cellmembranet och komma in i cellen. Signalering till GLUT4 kan ske via hormonet insulin men även medieras av fysisk aktivitet. Glykogen är ett viktigt substrat som utnyttjas för energiproduktion i samband med muskelarbete och vid slakt är muskulaturens innehåll av glykogen en viktig faktor som påverkar köttkvaliteten. Det finns olika former av glykogen (pro- och makroglykogen) som anses ha olika funktion och reglering i muskulaturen. Nedbrytning och återuppbyggning av pro- och makroglykogen sker på olika sätt i samband med muskelarbete. Målsättning med detta arbete var att studera skillnader mellan bärare och icke bärare av *PRKAG3* mutationen när det gäller fibertyper, enzymprofiler, resyntes av glykogen(pro- och makroglykogen) efter arbete på rullmatta och uttryck och fosforylering (aktivering) av signalproteiner involverade i insulin och icke insulin beroende glukosupptag. Grisar som var bärare eller inte bärare av mutationen utförde standardiserade fysiska arbeten på rullmatta före och efter en träningsperiod. Muskelprover (*m. biceps femoris*) och blodprover togs före och efter arbete samt i återhämtningsfasen. Vävnadsprover från *m.*

masseter, *m. biceps femoris, m. semitendinosus* och *m. longissimus dorsi* togs omedelbart efter avlivning. Bärare av mutationen hade jämfört med icke bärare högre glykogeninnehåll i alla provtagna muskler och framförallt var makroglykogen fraktionen högre. Efter 3 timmars återhämtning efter arbete var resyntesen, särskilt av makroglykogen, högre hos bärarna av mutationen. Detta sågs inte efter 6 timmars återhämtning. Det fanns inga skillnader mellan bärare och icke bärare när det gällde blodkoncentrationer av insulin, glukos, laktat och fria fettsyror efter arbete. Uttrycket av AMP kinas i muskeln var lägre hos bärare jämfört med icke bärare. Den ökade glykogensyntesen efter arbete kan korreleras med ett ökat Akt signaleringssvar och dess substrat AS 160 och högre hexokinasaktivitet, vilket tyder på ökat inflöde av glukos och fosforylering av glukos som styrs mot glykogensyntes. Immunohistokemiska metoder visade att bärare jämfört med icke bärare hade lägre procent typ IIB fibrer i *m. biceps femoris* . I *m. longissimus dorsi* hade bärare av mutationen en lägre procent typ IIB fibrer än icke bärare och dessutom en högre procent typ IIAX fibrer. I båda dessa muskler var den relativa arean av typ IIB fibrerna lägre hos bärare än hos icke bärare. *PRKAG3* mutationen påverkar muskelkaraktäristika hos tränade grisar och stimulerar en oxidativ fenotyp i varierad grad i muskler med olika funktioner.

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Acknowledgements

This PhD project was carried out at the Department of Clinical Sciences at the Swedish University of Agricultural Sciences (SLU). Funding was provided by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS).

This work was completed with the help of many people and I wish to express my gratitude to all of you. I wish to sincerely thank:

Birgitta Essén-Gustavsson, my main supervisor, for sharing your invaluable knowledge in muscle physiology. To me you are a true scientist and I have been fortunate to have you enthusiastically guiding me through this project. You are the only person that I know that can work without food and sleep and still come up with new brilliant ideas and conclusions. Time is endless…

Marianne Jensen-Waern, my co-supervisor, for introducing me to Lab Animal Science, providing a knowledge of pig models and for keeping me under your wings for so many years.

Kristina Karlström, for your excellent guiding in the lab. You have always taken the time to answer my questions and also supported me when something has not succeeded. I am very thankful for all analyses you have run. I think every lab should have a Kristina. I also would like to thank Anna Svensson and Marta Kot for all your help.

Bengt Norén for helping me with the pigs and also for being as fond of pigs as I am.

Jannica Krafft, Agneta Ljungqvist and Maria Nordqvist, all students who very ambitiously helped me with the training of the pigs and also named them.

Alexander Chibalin, Boubacar Benziane, Dana Galuska and Olga Kotova for collaboration, help and support with my work at Karolinska Institutet.

My room-mates, Märit Andersson, Robert Kruse, Patricia Hedenqvist and Elin Spangenberg, without you this would not have been possible. I really miss our discussions about science and life, but I am happy that we are able to keep in touch. All of you have made this journey enjoyable.

Katarina Cvek, Åsa Fahlman, Miia Riihimäki and Annette Backhans for entertaining company, support and interesting discussions. I also want to thank all other colleagues (present and former) at KV DOS for creating such a nice atmosphere.

Ulrika Hermansson, former fellow graduate student, for dog walks, support and friendship.

Kjell-Åke Alhin and Kristina Eriksson for computer and administrative help and caring.

Torgny Jeneskog, for friendship and encouragement throughout my thesis work.

My new colleagues at VetA, Karolinska Institutet, who have put up with me and my thesis so far.

To my parents for always loving and wanting to take care of our children.

Most of all, my beloved family: Johan for your love and wit and for keeping me focused on what really matters in life. I am also very thankful for all your help with my thesis even if you consider the academic world a place for them who do not fit in elsewhere. Karl, Gustav, Nils and Elsa for being my wonderful children.