Muscle glycogen depletion and resynthesis in highly trained male cyclists

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Tömning och återinlagring av muskelglykogen hos vältränade cyklister

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Abstract

Aim

The aim of this study was to establish a method to create a difference between groups in muscle glycogen content as well as to investigate the effect of training in low muscle glycogen state on metabolic and physiological parameters.

Method

During two trials, a subject group of ten highly trained male road or mountain bike cyclists ((mean±SD) age, hight, body weight, VO_2max, and VO_2max·kg^{-1} was 28±5 years, 74.7±6.3 kg, 183±6 cm, 4876±332 mL min^{-1}, 64.4±2.8 mL·kg^{-1} min^{-1}), performed a glycogen depletion exercise followed by a night's rest and a second exercise session. In the study, which was a crossover design, the subjects were randomly chosen to perform the first trial on a carbohydrate rich diet or a diet with no of carbohydrates. All the testing was performed on a Monark 839E ergometer bike and muscle biopsy sampling was collected before depletion exercise, before the exercise the following day and three hours post exercise. Plasma FFA and glucose was analyzed from venous blood collected at rest.

Results

Muscle glycogen pre depletion exercise was 623±180 and 645±133 mmol·kg dw^{-1} glycosyl units for non-CHO and CHO trials respectively. The depletion exercise followed by 13 hours of rest resulted in a significant decrease in muscle glycogen in the non-CHO ($p<0.0001$), and CHO trials ($p<0.01$) to 166±71 and 478±111 mmol·kg dw^{-1} respectively. In the non-CHO trial net glycogen depletion correlated positively with pre depletion glycogen storage. After the completion of exercise 2 and the following three hour rest period, glycogen content in non-CHO and CHO-trial was 130±52 and 477±97 mmol·kg dw^{-1}, respectively. In low glycogen state, the non-CHO trial resulted in an increase in FFA measured in blood plasma at rest and in an increase in Borg rating of perceived exertion (RPE) as well as a reduction in blood glucose during exercise.

Conclusion

The protocol used in the present study was successful in creating a difference in muscle glycogen storage and training in low glycogen state was associated with an increase of several physiological parameters indicating a possible impairment of endurance exercise performance.

Sammanfattning

Syfte

Syftet med denna studie var att skapa en metod för att åstadkomma skillnader i muskelglykogen samt observera den akuta effekten av träning med låga muskelglykogennivåer på metabola och fysiologiska parametrar.

Metod

Vid två tillfällen fick tio vältränade mountainbike- eller landsvägscyklister ((medel±SD) ålder, längd, kroppsvikt, VO_2max och VO_2max·kg^{-1} var 28±5 years, 74,7±6,3 kg, 183±6 cm, 4876±332 mL min^{-1}, 64,4±2,8 mL·kg min^{-1}) genomföra ett träningspass i syfte att tömma muskelglykogendepåerna följt av en natts vila och sedan ett andra träningspass. Studien följde ett randomiserat crossover-upplägg och det ena försökstillfället genomfördes med en diet hög på kolhydrater och det andra tillfället med en diet utan kolhydrater (CHO). All testning genomfördes på en Monark 839E ergometer och muskelbiopsier togs före tömningspass, efter en natts vila före det andra träningspasset och tre timmar efter det andra träningspasset. Venösa blodprov togs i vila före biopsitagning för analys av plasma FFA och glukos.

Resultat

Koncentrationen av muskelglykogen före tömningspasset var 623±180 and 645±133 mmol·kg dw^{-1} vid försök utan respektive med CHO. Tömningspasset och 13 timmars vila resulterade i en signifikant minskning av muskelglykogen vid försök utan CHO ($p<0.0001$), och med CHO ($p<0.01$) till 166±71 och 478±111 mmol·kg dw^{-1}. Nettominskningen av muskelglykogen vid tömningspasset utan CHO korrelerade positivt med glykogenkoncentration före tömning Efter genomförande av det andra träningspasset och tre timmars efterföljande vila var muskelglykogenmängden vid försöken utan CHO och med CHO 130±52 och 477±97 mmol·kg dw^{-1}. Vid träning med lågt muskelglykogen fanns det en kraftig ökning av fria fettsyror i blod vid vila och under arbete noterades en ökning skattning av Borg subjektivt skattad ansträngning (RPE) samt en sänkning av blodglukos.

Slutsats

Protokollet som användes i denna studie skapade framgångsrikt en minskning av muskelglykogen och träning med låga glykogennivåer kunde sammankopplas med flera fysiologiska parametrar som indikerar en möjlig sänkning av prestationsförmåga under uthållighetsarbete.

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1 Introduction

Muscle glycogen storage has been proven to be a determining factor for endurance capacity during high intensity exercise[1] and especially during endurance performances lasting longer than 1.5 h.[2] The relationship between muscle glycogen content and depletion during exercise is well documented and low glycogen content is closely linked to fatigue.[3] Muscle glycogen storage is thereby a key factor to optimise for maximizing performance ability. Also short-term muscle glycogen resynthesis after intense exercise has been investigated.[4] The ability to increase muscle glycogen content is a central question for performance during repeated exercise. Muscle glycogen resynthesis has been shown to be dependent on the previous type of exercise, nutritional timing and content[5] [6] [7], and various dietary supplements as caffeine[8] and various amino acids.[9] Also, the long term resynthesis of muscle glycogen over several days may be dependent on the timing of exercises undertaken by the individual.[10] Most of the existing studies to date have focused on optimising muscle glycogen storage or the resynthesis of muscle glycogen but little has been published with focus on muscle glycogen depleting protocols and resynthesis of muscle glycogen in absence of post exercise carbohydrate supplementation. With regard to the current attention to training with low or high muscle glycogen levels more knowledge is also needed regarding the individual capacity to exercise and perform under low glycogen condition with or without carbohydrate supplementation. Typically, when investigating the mitochondrial adaptation to endurance training of different

[1] HG Rauch. A St Clair Gibson. Lambert EV &TD Noakes, "A signalling role for muscle glycogen in the regulation of pace during prolonged exercise", *British Journal of Sports Medicine*, 39(2005:1), p. 4ff.
[2] JA Hawley, EJ Schabort, TD Noakes & SC Dennis, Carbohydrate-loading and exercise performance. An update *Sports Medicine*, 24(1997:2, Aug), p 73ff.
[3] J Bergström, Hermansen L, Hultman E & Saltin B, "Diet, muscle glycogen and physical performance", *Acta Physiologica Scandinavica*. 71(1967:2, Oct-Nov), p. 140ff.
[4] TJ Fairchild, S Fletcher, P Steele, C Goodman, B Dawson & PA Fournier, "Rapid carbohydrate loading after a short bout of near maximal-intensity exercise", *Medicine & Science in Sports & Exercise*, 34(2002:6, Jun), p. 980ff.
[5] DD Pascoe & LB Gladden, "Muscle glycogen resynthesis after short term, high intensity exercise and resistance exercise", *Sports Medicine*, 21(1996:2, Feb), p. 98-118.
[6] R Jentjens & A Jeukendrup, "Determinants of post-exercise glycogen synthesis during short-term recovery", *Sports Medicine*, 33(2003:2), p. 117-44.
[7] JL Ivy. AL Katz. CL Cutler, WM Sherman & EF Coyle, "Muscle glycogen synthesis after exercise: effect of time of carbohydrate ingestion", *Journal of Applied Physiology*, 64(1988:4, Apr), p.1480-5.
[8] DJ Pedersen. SJ Lessard. VG Coffey. EG Churchley. AM Wootton. MJ Ng T. Watt & JA Hawley, "High rates of muscle glycogen resynthesis after exhaustive exercise when carbohydrate is coingested with caffeine", *Journal of Applied Physiology*, 105(2008:1), p. 7-13.
[9] LJ van Loon, WH Saris, M Kruijshoop & AJ Wagenmakers, "Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures", *The American Journal of Clinical Nutrition*, 72(2000:1, Jul), p.106ff.
[10] P McInerney, SJ Lessard, LM Burke, VG Coffey, SL Lo Giudice, RJ Southgate & JA Hawley, "Failure to repeatedly supercompensate muscle glycogen stores in highly trained men.", *Medicine & Science in Sports & Exercise*, 37(2005:3, Mar), p.404-411.

training regimens or the acute effect of a single or numerous sets of exercise bouts in the presence of muscle glycogen, a method that can create a difference in muscle glycogen is needed. Previous research has in some cases failed to achieve a significant difference in muscle glycogen content between groups or has used a model that does not eliminate other explanations for the results than muscle glycogen levels (for review see Hawley & Burke). [11] The aim of the present study is therefore to create and evaluate a protocol for muscle glycogen depletion in highly trained cyclists. The protocol used in the present study is designed to generate data not only on muscle glycogen but also to provide an experimental protocol to investigate the effect of muscle glycogen on cell signalling for various transcription factors for mitochondrial biogenesis associated with adaptation to endurance training. Part of the training will take place in the presence or absence of stored muscle glycogen and the success of the method for creating differences in glycogen storage are thereby essential.

2 Background

Muscle glycogen is the primary energy substrate used during high intensity endurance exercise.[12] Scandinavian researchers concluded in the 1960s and 1970s that the size of muscle glycogen storage and the speed of endogenous muscle glycogen oxidation is a determinant for performance during endurance exercise and that dietary interventions can play a major part in optimising endurance performance.[13] The repletion of muscle glycogen in subjects who ingest a normal, balanced diet can occur as early as 24 hours post exercise[14], and during prolonged recovery also increase glycogen above baseline values, popularly referred to as glycogen super compensation.[15] It is well established that a carbohydrate (CHO) rich diet increases glycogen storage and that muscle glycogen super compensation is dependent on carbohydrate intake and timing.[16] Glycogen can be stored in two different forms: proglycogen (PG) and macroglycogen (MG) where MG has a higher amount of CHO per molecule. Human muscles with low muscle glycogen content contain approximately 1/4 of MG and as the subjects' total

[11] JA Hawley & LM Burke, "Carbohydrate availability and training adaptation: effects on cell metabolism", *Exercise and Sport Sciences Reviews*, 38(2010:4, Oct), p. 152ff.
[12] R Jentjens, 2003, p. 118.
[13] DA Sedlock, "The latest on carbohydrate loading: a practical approach", *Current Sports Medicine Reports*, 7(2008:4, Jul-Aug), p. 209.
[14] A Casey, AH Short, E Hultman & PL Greenhaff, "Glycogen resynthesis in human muscle fibre types following exercise-induced glycogen depletion", *Journal of Physiology*, 15(1995:1, Feb), p. 483.
[15] J Bergström & E Hultman "Muscle glycogen synthesis after exercise: an enhancing factor localized to the muscle cells in man", *Nature*, 210(1966:5033, Apr), p. 310.
[16] WM Sherman, JA Doyle, DR Lamb & RH Strauss, "Dietary carbohydrate, muscle glycogen, and exercise performance during 7 d of training", The American Journal of Clinical Nutrition, 57(1993:1, Jan), p. 29.

glycogen storage increases the percentage amount of MG increases.[17] As demonstrated by Adamo et. al. PG accounts for the rapid increase in glycogen after exercise whereas MG continues to increase over time if carbohydrate intake is sufficient.[18] The formation of MG has therefore been associated with the ability of muscle glycogen super compensation using a high CHO-diet.

When comparing different studies it is difficult to interpret the results because of the use of different methods to analyze glycogen, different biopsy sampling techniques and sites, different amount of glycogen storage and differences in training status of the subjects. A comparison between wet and dry muscle weight can be made by multiplying muscle glycogen wet weight ($mmol \cdot kg^{-1}$) by 4.28 to account for water weight as described by van Hall et. al.[19] In this paper all muscle glycogen values are expressed as dry weight.

2.1 Muscle glycogen synthesis: regulation and transport

The primary source of glucose in the post exercise state is ingested carbohydrate. Muscle glycogen resynthesis is limited at the cell membrane where the glucose is transported by facilitated diffusion. The two main expressed glucose transporter carrier proteins (GLUT) in skeletal muscle are GLUT-1 and GLUT-4.[20] The GLUT-4 isoform is located intracellularly in the muscle cell and is translocated to the membrane when insulin binds to its receptor[21] or by the stimulation of muscle contraction.[22] The maximal insulin simulated glucose uptake is however approximately 40% greater than by muscle contraction as demonstrated by Lund et. al.[23] The GLUT-1 isoform is located mostly at the membrane and appears to play a role in the non-insulin stimulated glucose transport[24]. The maximal rate of glucose transport is limited by the amount of GLUT-4 and its location in the muscle cell. The oxidative type I and type IIA muscle fibres are as a consequence of their composition more insulin sensitive, have a

[17] R Jentjens, 2003, p. 121.
[18] KB Adamo, MA Tarnopolsky & TE Graham, "Dietary carbohydrate and postexercise synthesis of proglycogen and macroglycogen in human skeletal muscle", *American Journal of Physiology*, 275(1998:2 pt 1, Aug), p. 232.
[19] G van Hall, SM Shirreffs & JA Calbet, "Muscle glycogen resynthesis during recovery from cycle exercise: no effect of additional protein ingestion", *Journal of Applied Physiology*, 88(2000:5, May), p. 1634.
[20] JL Ivy & CH Kuo, "Regulation of GLUT4 protein and glycogen synthase during muscle glycogen synthesis after exercise", *Acta Physiologica Scandinavica*, 162(1998:3, Mar), p. 295f.
[21] R Jentjens, 2003, p. 119.
[22] S Lund, GD Holman, O Schmitz & O Pedersen, "Contraction stimulates translocation of glucose transporter GLUT4 in skeletal muscle through a mechanism distinct from that of insulin", *Proceedings of the National Academy of Sciences*, 92(1995:13, Jun), p. 5820.
[23] Ibid, p. 5820.
[24] JL Ivy, 1998, p. 296.

greater GLUT-4 content and demonstrate a higher glucose transport capacity than fast twitch type IIB fibres.[25] Also, low muscle glycogen content has been proven to be a mediating factor affecting glucose transport by trigger translocation of the GLUT-4 isoform.[26] Muscle cell membrane permeability appears to be inversely related to muscle glycogen concentration during recovery and GLUT-4 translocation activity appears to decrease during recovery as muscle glycogen level increases.[27] Endurance exercise[28] and also hypoxia[29] has been proven to increase GLUT-4 protein and mRNA in skeletal muscle and thereby has an effect on muscle glucose transport. Studies in rat muscle have shown that GLUT-4 protein content and GLUT-4 mRNA increases after exercise. The combination of exercise and post exercise-fed carbohydrates have proven to further enhance this increase in GLUT-4 protein, but not in GLUT-4 mRNA expression[30]. In the same study, Kuo et. al. observed a positive correlation between absolute muscle glycogen content and GLUT-4 protein content ($r = 0.81$).[31] The effect of exercise on glucose transport can persist for several hours. Fell et. al. observed an increased glucose uptake in rat muscles when carbohydrates were infused with insulin after a 16 hour post-exercise fast compared to the control group that also ingested carbohydrates ad libitum after exercise. The fasted group showed a significantly higher glucose uptake at 16 hour post exercise as well as higher conversion of glucose to glycogen than the control group, though there was no difference in insulin levels between groups.[32]

2.1.1 The rapid and slow phase of glycogen synthesis – influencing factors

Muscle glycogen synthesis is often divided into two phases: the rapid and the slow phase. The first and rapid phase is insulin-independent and occurs during the first 30-60 minutes of recovery. The rapid phase is dependent on the availability of carbohydrate substrate[33] and also low muscle glycogen content. This has been demonstrated by Price et. al. by somatostatin

[25] PS MacLean, D Zheng & GL Dohm., "Muscle glucose transporter (GLUT 4) gene expression during exercise", *Exercise and Sport Sciences Reviews*, 28(2000:4, Oct), p. 148.
[26] R Jentjens, 2003, p. 119.
[27] JL Ivy, 1998, p. 299.
[28] A Zorzano, T Santalucia, M Palacín, A Gumà & M Camps, "Searching for ways to upregulate GLUT4 glucose transporter expression in muscle", *General Pharmacology*, 31(1998:5, Nov), p. 715.
[29] EA Richter, W Derave & JF Wojtaszewski, "Glucose, exercise and insulin: emerging concepts", *Journal of Physiology*, 1:535(2001:2, Sep), p. 535.
[30] CH Kuo, DG Hunt, Z Ding & JL Ivy, "Effect of carbohydrate supplementation on postexercise GLUT-4 protein expression in skeletal muscle", *Journal of Applied Physiology*, 87(1999:6, Dec), p. 2293.
[31] Ibid, p. 2293.
[32] RD Fell, SE Terblanche, JL Ivy, JC Young & JO Holloszy, "Effect of muscle glycogen content on glucose uptake following exercise", *Journal of Applied Physiology*, 52(1982:2, Feb), p. 436.
[33] R Jentjens, 2003, p. 121.

infusion to inhibit pancreatic insulin secretion during post exercise recovery.[34] The second and slow phase takes place in the presence of insulin and fed carbohydrates during the following hours after exercise.[35] A negative correlation of glucose transport rate and GLUT-4 cell surface content to glycogen levels in both contracting and resting fast twitch rat muscle has also been observed[36] which explains the high non insulin stimulated glucose transport observed after exercise.

The rapid phase of muscle glycogen synthesis has also been suggested to be highly influenced by glycogen synthase, an enzyme that is considered to be the main rate limiting enzyme in the process of transforming glucose to glycogen. Glycogen synthase exists in the muscle in an active non phosphorylated form (I) and in several inactive phosphorylated forms (D).[37] The conversion of glycogen synthase D-form to I-form is mediated by muscle contraction, low glycogen levels and high insulin levels. [38, 39] Low muscle glycogen content has previously been observed to be reversely correlated to muscle glycogen content[40] and has been suggested by Nielsen et. al. to be the prime mediator of glycogen synthase activity.[41]

During the slow phase of muscle glycogen synthesis, increased insulin sensitivity can persist for over 48 hours when carbohydrates are fed and glycogen levels have not reached maximum.[42] Even a single bout of exercise and also continuously over a period of time previously performed exercise results in increased insulin sensitivity. The increased insulin sensitivity resulting from these factors is partly unexplained, but the main mediating factor

[34] TB Price, DL Rothman, R Taylor, MJ Avison, GI Shulman & RG Shulman, "Human muscle glycogen resynthesis after exercise: insulin-dependent and -independent phases", *Journal of Applied Physiology,* 76(1994:1 Jan), p. 106.
[35] JL Ivy, 1988, p. 1481f.
[36] W Derave, S Lund, GD Holman, J Wojtaszewski, O Pedersen & EA Richter, "Contraction-stimulated muscle glucose transport and GLUT-4 surface content are dependent on glycogen content", *American Journal of Physiology*, 277(1999:6 pt 1 Dec), p. 1107f.
[37] JJ Zachwieja, DL Costill, DD Pascoe, RA Robergs & WJ Fink, "Influence of muscle glycogen depletion on the rate of resynthesis", *Medicine & Science in Sports & Exercise,* 23(1991:1, Jan), p. 44.
[38] R Jentjens, 2003, p.122.
[39] E Montell, A Arias & AM Gómez-Foix, "Glycogen depletion rather than glucose 6-P increments controls early glycogen recovery in human cultured muscle", *American Journal of Physiology*, 276(19995 pt 2, May), p. 1491.
[40] JJ Zachwieja, p. 46.
[41] JN Nielsen, W Derave, S Kristiansen, E Ralston, T Ploug & EA Richter, "Glycogen synthase localization and activity in rat skeletal muscle is strongly dependent on glycogen content", *Journal of Physiology*, 531(2001:3, Mar), p. 763f.
[42] R Jentjens, 2003, p. 123.

appears to be glycogen storage, which is shown by the decrease in insulin sensitivity as a result of an increase in glycogen storage.[43]

Muscle glycogen depletion appears to be an important mediating factor of glycogen synthesis rates.[44] Several studies have demonstrated that muscle glycogen depletion has a positive effect on glycogen synthesis rates[45,46] and that the effect appears to be related to the absolute low level of remaining glycogen and not the magnitude of the depletion[47]. There appears to be no difference in glycogen synthesis rate when consuming carbohydrates in liquid or solid form[48]. It has been proposed that the ingestion of smaller portions of carbohydrates at more frequent intervals is advantageous.[49] For example, Doyle et. al. presented high glycogen synthesis rates of 35-47 $mmol \cdot kg^{-1} \cdot h^{-1}$ in a study using short interval CHO-feeding every 15 minutes[50]. The synthesis rates were high but there was no comparison to less frequent CHO-feeding in that study making the results hard to interpret. As concluded by Jentjens et. al. short term feeding of post exercise carbohydrate appears to have no advantageous affect as long as the total amount of carbohydrates is sufficient.[51] High glycemic index value (GI) of the carbohydrates fed after exercise appears to be the best choice in order to achieve high synthesis rates during recovery.[52] It has been debated whether the gastric emptying process is a limiting factor for glucose uptake in muscular tissue and extremely high values of glycogen synthesis rates of more than 150 $mmol \cdot kg\ dw^{-1} \cdot h^{-1}$ have been reported by Hansen et. al. using glucose/insulin infusion during eight hours of recovery.[53] This indicates that the digestion/absorbing process of CHO is partly limiting in the resynthesis process, but in studies comparing gastric emptying and exogenous CHO oxidation of orally fed CHO at different

[43] LB Borghouts & HA Keizer, "Exercise and insulin sensitivity: a review", *International Journal Sports Medicine*, 21(2000:1 Jan), p. 2ff.
[44] R Jentjens, 2003, p. 135.
[45] A Bonen, GW Ness, AN Belcastro & RL Kirby, "Mild exercise impedes glycogen repletion in muscle", *Journal of Applied Physiology*, 58(1985;5, May), p. 1623.
[46] JJ Zachwieja, p. 46.
[47] TB Price, D Laurent, KF Petersen, DL Rothman & GI Shulman, "Glycogen loading alters muscle glycogen resynthesis after exercise", *Journal of Applied Physiology*, 88(2000:2, Feb), p. 700ff.
[48]MJ Reed, JT Jr Brozinick, MC Lee & JL Ivy, "Muscle glycogen storage postexercise: effect of mode of carbohydrate administration", *Journal of Applied Physiology,* 66(1989:2, Feb), p. 722.
[49]R Jentjens, 2003, p. 135.
[50] JA Doyle, WM Sherman & RL Strauss, "Effects of eccentric and concentric exercise on muscle glycogen replenishment", *Journal of Applied Physiology*, 74(1993:4, Apr), p. 1852.
[51] R Jentjens, 2003, p. 135.
[52] Ibid, p. 133.
[53] BF Hansen, S Asp, B Kiens & EA Richter, "Glycogen concentration in human skeletal muscle: effect of prolonged insulin and glucose infusion", *Scandinavian Journal of Medicine & Science in Sports*, 9(1999:4, Aug), p. 211.

gastric emptying rates, gastric emptying rate is not the limiting factor. [54] Orally fed CHO will far from completely end up as muscle glycogen. It is partly oxidised, used to restore liver glycogen or is synthesised to fat in previously exercised muscles.[55] Bowtell et. al. observed whole body absorption of ingested CHO during two hours of recovery after ingestion of a 18.5% glucose polymer drink (78%) and a 18.5% sucrose drink (42 % absorption after 2 hour, respectively). The absorption of ingested carbohydrates was not due to differences in gastric emptying speed and the authors concluded that the insulinogenic property of the fructose component in sucrose was the determining factor for whole body absorption.[56]

Further more, there appears to be no difference in glycogen synthesis after concentric or eccentric exercise during the early hours of recovery.[57] However, eccentric exercise might impair glycogen synthesis during the latter stage of recovery (18-72 h post exercise) which, interestingly, is the same period when inflammatory cell response to muscle damage occurs. [58] Environmental influences might also have an effect on synthesis rates. In a recent study an impairment of glycogen synthesis during post exercise recovery was observed when environmental conditions were altered and the room temperature was set to 32 ºC. Glycogen synthesis rates were significantly lower during 2-4 hours post exercise when the subjects rested under hot conditions, resulting in a significant increase in core temperature, compared to recovery in a normal ambient temperature of 22 ºC. [59]

2.1.2 Fibre type composition and glycogen synthesis

Most of the studies concerning glycogen restoration are performed on mixed fibre samples from biopsies or have used magnetic resonance scanning (MRS). A few studies, however, have with mixed results performed single fibre analysis to detect fibre type differences on muscle glycogen synthesis.[60] Casey et. al. showed after a one leg sub maximal exercise to exhaustion that glycogen synthesis rates in type I fibres were higher than in type II fibres during 1-3 hour of recovery. During 3-10 hours the synthesis rates increased in type II fibres and were significantly higher than in type I fibres. During 10-24 hours there was no difference

[54] R Jentjens, 2003, p. 135.
[55] Ibid, p. 138.
[56] JL Bowtell, K Gelly, ML Jackman, A Patel, M Simeoni & MJ Rennie, "Effect of different carbohydrate drinks on whole body carbohydrate storage after exhaustive exercise", *Journal of Applied Physiology*, 88(2000:5, May), p. 1534.
[57] JA Doyle, p. 1852.
[58] R Jentjens, 2003, p. 137.
[59] M Naperalsky, B Ruby & D Slivka, "Environmental temperature and glycogen resynthesis", *International Journal Sports Medicine*, 31(2010:8, Aug), p. 563.
[60] R Jentjens, 2003, p. 136.

between fibre types in the rate of glycogen resynthesis.[61] Muscle glycogen concentration of the specific fibre appears to be closely related to synthesis rates. One reason for the initial increase in synthesis rate in type I fibres could be the higher content of GLUT-4 in type I fibres, which have previously been found to correlate positively to synthesis rates.[62] The results from different studies are difficult to compare due to the varying protocols used to deplete glycogen. In studies using low intensity endurance workout, the depletion will be larger in type I fibres[63] and in studies involving high intensity interval or resistance training, it has been suggested that the re-conversion of muscle lactate will contribute to higher synthesis rates during recovery.[64] During active rest using low intensity exercise it is shown in rats that the replenishment of muscle glycogen is enhanced in fast twitch type II fibres but reduced in slow twitch type I fibres.[65] Higher synthesis rates have also been observed in type II fibres than in type I fibres when high intensity intervals were added to the latter period of a depleting exercise protocol, probably due to increased glucose transport stimulated by fibre specific low glycogen content.[66] There is also evidence that GLUT-4 protein expression increases as a consequence of training without CHO-supplementation, indicating a long term training effect resulting in an increased capacity of glycogen resynthesis.[67]

2.2 CHO intake: timing and amount

There is an advantage in consuming carbohydrates as close as possible to the end of exercise since the glycogen transport and synthesis is increased as a result of exercise. As demonstrated by Ivy et. al. muscle glycogen synthesis is impaired during fasting. When carbohydrates were withheld during the first two hours of recovery glycogen synthesis rates were significantly lower in comparison to when CHO was supplemented at end point of exercise. After being fed carbohydrates, the delayed CHO-group showed an increase in

[61] A Casey, p. 483.
[62] RC Hickner, JS Fisher, PA Hansen, SB Racette, CM Mier, MJ Turner & JO Holloszy, "Muscle glycogen accumulation after endurance exercise in trained and untrained individuals", *Journal of Applied Physiology*, 83(1997:3, Sep), p. 899.
[63] K De Bock, W Derave, M Ramaekers, EA Richter & P Hespel, "Fiber type-specific muscle glycogen sparing due to carbohydrate intake before and during exercise", *Journal of Applied Physiology*, 102(2007:1 Jan), p. 186.
[64] J Bangsbo, PD Gollnick, TE Graham & B Saltin, "Substrates for muscle glycogen synthesis in recovery from intense exercise in man", *Journal of Physiology*, 434(1991, Mars), p. 429.
[65] G Raja, L Bräu, TN Palmer & PA Fournier, "Fiber-specific responses of muscle glycogen repletion in fasted rats physically active during recovery from high-intensity physical exertion", *American Journal of Physiology Regulative, Integrative and Comparative Physiology*, 295(2008:2, Aug), p. 635.
[66] K Piehl, "Time course for refilling of glycogen stores in human muscle fibres following exercise-induced glycogen depletion", *Acta Physiologica Scandinavica*, 90(1974:2, Feb), p. 297.
[67] L Nybo, K Pedersen, B Christensen, P Aagaard, N Brandt & B Kiens, "Impact of carbohydrate supplementation during endurance training on glycogen storage and performance", *Acta Physiologica Scandinavica, (Oxf)*, 197(2009:2, Oct), p. 121.

plasma glucose, glycogen synthase activity and glycogen synthesis rate. The delayed CHO-group thereby had an increase in glycogen synthesis but as a result of the delay in ingestion net glycogen content was lower after four hours of recovery.[68] If no carbohydrates are consumed after exercise glycogen synthesis rates are low. Typical values presented by van Hall et. al. are glycogen synthesis rates of 18 mmol·kg dw^{-1}·h^{-1} during the first 1.5 hours of recovery and 8 mmol·kg dw^{-1}·h^{-1} for the remainder of a four hour long rest.[69] These values are in order with previous presented values of 7-12 mmol·kg dw^{-1}·h^{-1} summarized in a review by Jentjens and Jeukendrup.[70]

When CHO is fed orally after exercise, general rates of 20-50 mmol·kg dw^{-1}·h^{-1} have been reported in several studies.[71] The difference in glycogen synthesis rates in different studies could be explained by several factors as differences in glycogen depletion, experimental protocol, training status of the subjects, the type of CHO supplemented and differences in biopsy sampling. The effect of different amounts of CHO has been tested in several studies. Ivy et. al. reported no significant difference in synthesis rate when 0.75 and 1.5 g·kg^{-1}·h^{-1} was ingested in two hour intervals after exercise during four hours of recovery (19.6 versus 22.0 mmol·kg dw^{-1}·h^{-1}).[72] Similar synthesis rates were reported by Bloom et. al. when glucose intake was elevated from 0.35 to 0.7 g·kg^{-1}·h^{-1} in two hour intervals between feedings (24.8 vs. 24.4 mmol·kg dw^{-1}·h^{-1}).[73] In a study by van Loon glycogen synthesis rates were increased to 44.8 mmol·kg dw^{-1}·h^{-}1 when 1.2 g·kg^{-1}·h^{-1} was consumed at 30-minute intervals compared to 16.6 mmol·kg dw^{-1}·h^{-1} when only 0.8 g·kg^{-1}·h^{-1} was ingested. [74] However, the difference in timing of the post exercise fed CHO should not be a factor that solely explains the results[75], nor does there appear to be a significant difference in the usage of liquid or solid form CHO.[76] Previous investigation by Costill et. al. indicates higher glycogen storage and synthesis rates after a high CHO diet in comparison to a low CHO diet[77] and as summarized in review by

[68] JL Ivy, 1988, p. 1481.
[69] G van Hall, p. 1634.
[70] R Jentjens, 2003, p. 124.
[71] Ibid, p. 124.
[72] JL Ivy, MC Lee, JT Jr Brozinick & MJ Reed, "Muscle glycogen storage after different amounts of carbohydrate ingestion", *American Journal of Physiology*, 65(1988:5, Nov), p. 2019.
[73] PC Blom, AT Høstmark, O Vaage, KR Kardel & S Maehlum, "Effect of different post-exercise sugar diets on the rate of muscle glycogen synthesis", *Medicine & Science in Sports & Exercise*, 19(1987:5, Oct), p. 493.
[74] LJ van Loon, 2000, p. 109.
[75] R Jentjens, 2003, p. 130.
[76] MJ Reed, JT, p. 722.
[77] DL Costill, WM Sherman, WJ Fink, C Maresh, M Witten & JM Miller, "The role of dietary carbohydrates in muscle glycogen resynthesis after strenuous running", *The American Journal of Clinical Nutrition*, 34(1981:9, Sep), p. 1833.

Jentjens and Jeukendrup there seems to be a positive correlation between muscle glycogen synthesis rate and the amount of CHO up to 1.5 $g \cdot kg^{-1} \cdot h^{-1}$.[78] Some studies have failed to show an increase in muscle glycogen resynthesis during recovery after a dietary intervention. For example, Reinert et. al. concluded that a 240 kcal CHO + protein drink served 30 minutes after two hours of low/medium intensity cycling exercise did not have an affect on synthesis rates compared to a placebo drink when CHO was fed before/during exercise as well as a solid meal two hours post exercise.[79]

2.3 Intake of CHO in presence with other nutrients

To maintain high glycogen synthesis rates plasma insulin levels must be stimulated to remain elevated during recovery. Several studies have examined the contributing effect of different substances such as protein, amino acids, CHO + fat[80] and caffeine [81] in attempt to increase insulin levels and glycogen synthesis rates. When carbohydrates (0.8 $g \cdot kg^{-1} \cdot h^{-1}$) were combined with amino acids + whey protein, van Loon et. al. reported improved synthesis rates compared to carbohydrate intake alone during five hours of recovery (CHO 16.6 and 35.4 $mmol \cdot kg\ dw^{-1} \cdot h^{-1}$ respectively).[82] Likewise, Zawadzki et. al. observed increased synthesis rates of 38% when carbohydrates were combined with proteins.[83] The different effect of various amino acids on insulin response have also been tested and verified by van Loon.[84] When investigated by Jentjens et. al. if a higher supplemented dose of carbohydrates of 1.2 $g \cdot kg^{-1} \cdot h^{-1}$ in combination with amino acids resulted in higher glycogen synthesis rates the difference in synthesis rates to carbohydrates alone was not significant, although there was a significant increase in plasma insulin when CHO was combined with amino acids.[85] Muscle glycogen synthesis rates appear to be stimulated by amino acids when the supplemented carbohydrate dose is lower than the maximal uptake rate but the differences diminish when

[78] R Jentjens, 2003, p. 138.
[79] A Reinert, D Slivka, J Cuddy & B Ruby, "Glycogen synthesis after road cycling in the fed state", *International Journal Sports Medicine*, 30(2009:7, Jul), p. 547.
[80] JL Ivy, HW Jr Goforth, BM Damon, TR McCauley, EC Parsons & TB Price,"Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement", *Journal of Applied Physiology*, 93(2002:4, Oct), p. 1340.
[81] DJ Pedersen, p. 7.
[82] LJ van Loon, 2000, p. 109.
[83] KM Zawadzki, BB 3rd Yaspelkis & JL Ivy, "Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise", *Journal of Applied Physiology*, 72(1992:5, May), p. 1856.
[84] LJ van Loon, WH Saris, H Verhagen & AJ Wagenmakers, "Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate", *The American Journal of Clinical Nutrition*, 72(2000:1, Jul), p. 103.
[85] RL Jentjens, LJ van Loon, CH Mann, AJ Wagenmakers & AE Jeukendrup, "Addition of protein and amino acids to carbohydrates does not enhance postexercise muscle glycogen synthesis", *Journal of Applied Physiology*, 91(2001:2, Aug), p. 842.

CHO intake increase above 1.2 $g \cdot kg^{-1} \cdot h^{-1}$.[86] During long term recovery up to 24 hours there appears to be no difference in restored glycogen content when CHO is combined with protein and fat in a mixed diet or is eaten alone as long as the CHO amount is sufficient.[87] Caffeine has been proven to increase both exogenous carbohydrate oxidation during exercise[88] and glycogen synthesis during recovery.[89] Pedersen et. al. have reported an average muscle glycogen resynthesis of 60 $mmol \cdot kg\ dw^{-1} \cdot h^{-1}$ during four hours of recovery when carbohydrates were fed orally in addition with caffeine.[90] These are among the highest reported values of glycogen resynthesis. It has also been shown by Battram et. al. that muscle glycogen resynthesis after intake of CHO + caffeine peaked at 30 minutes of recovery at 72 $mmol \cdot kg\ dw^{-1} \cdot h^{-1}$, then declined during the following 90 minutes and then stabilized during the remainder of recovery – resulting in an overall net resynthesis of 50 $mmol \cdot kg\ dw^{-1} \cdot h^{-1}$.[91]

2.4 Muscle glycogen depletion

Exercise in order to reduce muscle glycogen has long been a part of a regimen in order to maximize muscle glycogen storage. It has been demonstrated by Gofort et. al. that a CHO-loading protocol that begins with a depletion exercise increases muscle glycogen resynthesis in comparison to a high CHO-diet. al.one. [92] The depletion exercise does not only improve the speed of glycogen resynthesis but also the total amount of muscle glycogen that can be stored. The author also concluded that light daily exercise of 20 minutes at 65% of VO_{2max} had no a negative effect on muscle glycogen super compensation. [93] A large inter-subject variability (CV of 43%) in muscle glycogen content at exhaustion has been reported [94] and also demonstrated in numerous studies that muscle glycogen content never reaches zero at voluntarily exhaustion. Demonstrated by Rauch et. al. in well trained cyclists that were supplemented with either a CHO-rich or a normal diet the days preceding exercise. Post

[86] LJ van Loon, 2000, p. 109.
[87] LM Burke, GR Collier, SK Beasley, PG Davis, PA Fricker, P Heeley, K Walder & M Hargreaves. "Effect of coingestion of fat and protein with carbohydrate feedings on muscle glycogen storage", *Journal of Applied Physiology*, 76(1995:6, Jun), p . 2188.
[88] SE Yeo, RL Jentjens, GA Wallis & AE Jeukendrup, "Caffeine increases exogenous carbohydrate oxidation during exercise", *Journal of Applied Physiology*, 99(2005:3, Sep), p. 846.
[89] DJ Pedersen, p. 7f.
[90] Ibid , p. 10.
[91] DS Battram, J Shearer, D Robinson & TE Graham, "Caffeine ingestion does not impede the resynthesis of proglycogen and macroglycogen after prolonged exercise and carbohydrate supplementation in humans", *Journal of Applied Physiology*, 96(2004:3, Mar), p. 946.
[92] HW Jr Goforth, D Laurent, WK Prusaczyk, KE Schneider, KF Petersen & GI Shulman, "Effects of depletion exercise and light training on muscle glycogen super compensation in men", *American Journal of Physiology - Endocrinology and Metabolism*, 285(2003:6, Dec), p. 1307ff.
[93] Ibid, p. 1307ff.
[94] HG Rauch, p. 35f.

exercise muscle glycogen after completing 2 hour at 73% of VO_2max plus an additional one hour time trial to exhaustion was 85.6 ± 13 and 77 ± 13 mmol·kg dw^{-1}·h^{-1}. The remaining muscle glycogen content at the end of exercise differed between the individuals but was almost identical in the two conditions on an individual level, although there was a remarkable difference in muscle glycogen storage at the start of exercise as a result of the dietary intervention. [95]

The magnitude and pattern of muscle glycogen depletion is fibre type specific and dependent on duration and intensity of the exercise. As demonstrated by Gollnick et. al. in well trained subjects during endurance cycling exercise at 31, 64 and 83% of VO_2max ST fibres were gradually depleted over time during trial at 31% of VO_2max but the glycogen content of FT fibres was almost unchanged. The 64% intensity resulted in a more rapid reduction of glycogen content in ST fibres and a pronounced increase in FT fibre depletion during the latter stage of exercise when ST fibres were almost completely depleted. The 83% trial displayed the same pattern of fibre type depletion as the 64% trial but at a more rapid speed of glycogen depletion. During a high intensity interval session at an intensity corresponding to energy exchange equivalent to 120 and 150% of VO_2max (3 min 120%, 1 min 150% and 10 min rest – repeated until exhaustion) the depletion rate was rapid and to the same extent in both ST and FT fibres.[96] Suriano et. al. observed a negative relationship between glycogen depletion in type-II fibres and percentage of type-I fibres during constant intensity cycling at 90% of lactate threshold (LT) in well trained triathletes. The same group also conducted a second trial with the same amount of work performed but in an incremental form mixing 5 minute intervals at 70/110 % of LT. The mixed intensity trial resulted to a larger extent of depletion in type-II fibres.[97] Similar results after continuous low intensity exercise have been observed by Krustrup et. al. after a 3-hour depletion cycling exercise at 40% of VO_2max followed by a 15 hour fast. Slow twitch fibres were depleted to a much greater extent than FTa and FTx fibres.[98] In summary, a successful depletion protocol should include a variety of different intensities in order to activate all fibre types.

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[95] Ibid, p 35f.
[96] PD Gollnick, K Piehl & B Saltin,"Selective glycogen depletion pattern in human muscle fibres after exercise of varying intensity and at varying pedalling rates", *Journal of Physiology*, 241(1974:1, Aug), p.50ff.
[97] R Suriano, J Edge & D Bishop, "Effects of cycle strategy and fibre composition on muscle glycogen depletion pattern and subsequent running economy, *British Journal of Sports Medicine*, 44(2010:6, May), p. 445f.
[98] P Krustrup, K Söderlund, M Mohr & J Bangsbo,"Slow-twitch fiber glycogen depletion elevates moderate-exercise fast-twitch fiber activity and O2 uptake", *Medicine & Science in Sports & Exercise*, 36(2004:6, Jun), p.976.

2.5 CHO loading and exercise

The often presented CHO-loading protocol stretches over several days which is not always practical during competition preparation. Strategies for enhancing glycogen synthesis to stimulate supramaximal glycogen levels have therefore been presented. Fairchild et. al. demonstrated that a near maximal intensity exercise of not more than 3 minutes in addition to a 24 hour CHO loading protocol resulted in supranormal muscle glycogen content. The glycogen synthesis was also apparent in all fibre types.[99] Although the effect of enhanced capacity to store muscular glycogen after a single bout of exercise has been well studied, there is less knowledge about glycogen resynthesis during periods of frequent training resulting in frequent depletion of glycogen storage. During a five-day period including three depletion exercises and the use of a high carbohydrate diet, McInerney et. al. observed a super compensation in glycogen storage after the first depletion exercise but not after the second. Short-term glycogen synthesis was not different during the three trials but there was a marked difference in end glycogen storage between trials although carbohydrate intake was sufficient and there was no significant difference in exercise induced glycogen depletion between trials.[100] Long-term glycogen storage has also been tested using an extremely carbohydrate rich diet. After three weeks of diet and daily training the muscle glycogen was higher in the 88 E% CHO-group than in subjects fed with 68 or 58 E% CHO. As a result of the 88 E% CHO-diet, intramuscular triglyceride content and fat oxidation during exercise was dramatically reduced.[101] It has been proposed that there is a gender difference in response to carbohydrate feeding. Regarding gender differences in the ability to store muscle glycogen there is little evidence that the practical advice based on studies using male subjects should not be used on women. Tarnopolsky, et. al suggested that there could be gender differences in the ability of glycogen super compensation. In the study, male subjects increase their muscle glycogen by 41% after four days of a high carbohydrate diet (73 E% vs. 59 E% from control) whereas the women did not increase muscle glycogen at all from pre values.[102] However, the study has been criticized for supplementing too small amounts of CHO to the female subjects.

[99] TJ Fairchild, p. 982f.
[100] P McInerney, p.407ff.
[101] EF Coyle, AE Jeukendrup, MC Oseto & BJ Hodgkinson, TW Zderic "Low-fat diet alters intramuscular substrates and reduces lipolysis and fat oxidation during exercise", *American Journal of Physiology - Endocrinology and Metabolism*, 280(2001:3, Mar), p. 392ff.
[102] MA Tarnopolsky, SA Atkinson, SM Phillips & JD MacDougall, "Carbohydrate loading and metabolism during exercise in men and women, *Journal of Applied Physiology*, 78(1995:4, Apr), p. 1365.

It has been demonstrated that trained endurance athletes have, as a result of training, increased insulin sensitivity, glycogen synthase activity, increased GLUT-4 protein concentration and increased blood flow in comparison to sedentary subjects.[103] In a study by Greiwe et. al. GLUT-4 content increased twofold and muscle glycogen storage increased nearly twofold measured after 15 min, 6h and 48 of recovery after the subjects had completed a ten weeks training program.[104] The results are supported by previous results by Hickner et. al. who reported an increased ability for endurance trained subjects to restore muscle glycogen compared to sedentary subjects during 72 hours of recovery (at 6 h 303 vs. 131, at 48 h 701 vs. 423 and at 72 h 778 vs. 624 mmol·kg dw^{-1} respectively). In the study, increased glycogen content correlated with GLUT-4 content and also percentage of type I fibres.[105] The increase in GLUT-4 content appears to be tightly connected to training status, as the GLUT-4 protein appears to decrease rapidly during prolonged recovery indicating a short half-life of approximately ten hours of the GLUT-4 protein.[106]

Finally, it is worth mentioning the limitation in the ability to use muscle glycogen stored in non-working muscles as energy substrate in working muscles. It has been demonstrated during a one leg exercise that glycogen content in the resting leg does not contribute to the energy expenditure in the working leg during exercise.[107] Although during exercise, muscles with low glycogen content appear to have an increased lactate uptake converting lactate to pyruvate, which is used as energy substrate.[108]

2.6 Manipulation of muscle glycogen content

Training with low muscle glycogen content has lately been proposed to have a stimulatory affect on adaption to endurance training and several studies has been completed in aiming to

[103] P Ebeling, R Bourey, L Koranyi, JA Tuominen, LC Groop, J Henriksson, M Mueckler, A Sovijärvi & VA Koivisto, "Mechanism of enhanced insulin sensitivity in athletes. Increased blood flow, muscle glucose transport protein (GLUT-4) concentration, and glycogen synthase activity", *The Journal of Clinical Investigation*, 92(1993:4, Oct, p. 1627.
[104] JS Greiwe, RC Hickner, PA Hansen, SB Racette, MM Chen & JO Holloszy, "Effects of endurance exercise training on muscle glycogen accumulation in humans, *Journal of Applied Physiology*, 87(1999:1, Jul), p. 224.
[105] RC Hickner, p. 898f.
[106] HH Host, PA Hansen, LA Nolte, MM Chen & JO Holloszy, "Rapid reversal of adaptive increases in muscle GLUT-4 and glucose transport capacity after training cessation", *Journal of Applied Physiology*, 84(1998:3, Mar), p.
[107] J Bergström & E Hultman, A study of the glycogen metabolism during exercise in man, *Scandinavian Journal of Clinical and Laboratory Investigation*, 19(1967;3), p. 221.
[108] H Howard, JR Poortmans. B Essen, B Pernow, PD Gollnick & B Saltin, Muscle glycogen content and lactate uptake in exercising muscles.In Metabolic Adaptations to Prolonged Physical Exercise, eds Howard H, Poortmans JR. (Birkhauser, Basel), (1975), p. 133.

investigate the acute and long term adaption to training in low glycogen-state. [109]In these studies a difference in glycogen content between groups is achieved by the usage of a deplete/reload model. A depletion exercise is performed followed by a second exercise session undertaken after a period of rest where no carbohydrates are supplemented, or after a longer period of time or when a normal or high CHO-diet is provided. As an example, Yeo et. al. have used a depletion protocol where the subjects cycled at 70% of VO_2max for 100 minutes and after one hour of rest completed the next exercise in low glycogen state. The same protocol was used in two studies and the second exercise session were in the low-group performed with a glycogen content corresponding to ~ 65 % and ~ 70 % of the glycogen content of the high-group.[110] [111]. The mentioned protocols as well as previous research[112] have separated the second exercise sessions from the depleting exercise with a day of recovery to restore glycogen content in high CHO-state. This meaning that besides a comparison of training in high or low condition, an actual comparison of training twice a day or every second day is made. In studies avoiding this methodological bias the amount of work performed in the different states might differ between conditions. For example, Churchley et. al. manage to get muscle glycogen in low trial reduced to ~ 44% of high trial. [113] The study used a combination of various exercises including one-legged cycling to reduce muscle glycogen in the evening before the low/high –trial the following morning. The different legs were categorized as high and low depending on which leg that had performed one-legged cycling the previous evening and thereby had performed different amount of exercise prior to trial which might had an effect on the results.

3 Aim

The aim of this study was to establish a method to create differences between groups in muscle glycogen content as well as observing the resynthesis of muscle glycogen in subjects fed with either a carbohydrate rich diet or a diet completely absent of carbohydrates. The aim

[109] AK Hansen, CP Fischer, P Plomgaard, JL Andersen, B Saltin & BK Pedersen, "Skeletal muscle adaptation: training twice every second day vs. training once daily". *Journal of Applied Physiology*, 98(2005:1), p. 93-99.
[110] WK Yeo, SL McGee, AL Carey, CD Paton, AP Garnham, M Hargreaves & JA Hawley, "Acute signalling responses to intense endurance training commenced with low or normal muscle glycogen", *Experimental Physiology*, 95(2009:2), p. 354.
[111] WK Yeo, CD Paton, AP Garnham, LM Burke, AL Carey & JA Hawley, "Skeletal muscle adaptation and performance responses to once a day versus twice every second day endurance training regimens", *Journal of Applied Physiology*, 105(2008:5), p. 1465.
[112] AK Hansen, p. 96.
[113] EG Churchley, VG Coffey, DJ Pedersen, A Shield, KA Carey, D Cameron-Smith & JA Hawley, "Influence of preexercise muscle glycogen content on transcriptional activity of metabolic and myogenic genes in well-trained humans", *Journal of Applied Physiology*, 102(2007:4), p.1606.

was also to investigate the effect of training with different muscle glycogen levels on the exercise-induced response in blood glucose, blood lactate, heart rate and rate of perceived exhaustion (RPE).

3.1 Hypothesis

1. With a combination of exercise and nutrition a significant difference in muscle glycogen content can be achieved after 13 hours of rest.

2. Exercise with low muscle glycogen will result in significant differences in metabolic and physiologic response during exercise compared to exercise with high muscle glycogen.

4 Methods

4.1 Subjects and preliminary testing

All subjects signed a health form and gave their written consent to participate in the present study that was approved by the regional ethical review board in Stockholm and was designed according to the declaration of Helsinki. The subject group was comprised of ten highly trained male road or mountain bike cyclists. They were all active competitors at a national elite level or had been competing at a national elite level during the preceding years. Mean±SD age, higth, body weight, VO_2max,VO_2max·kg^{-1}, W_{max} and W_{max}·kg^{-1} was 28±5 years, 74.7±6.3 kg, 183±6 cm, 4876±332 mL min^{-1}, 64.4±2.8 mL·kg^{-1} min^{-1}, 387±25 W_{max} and 5.21±0.42 W_{max}·kg^{-1}. All testing was performed on a Monark 839E ergometer bike (Monark Exercise, Varberg, Sweden) and held at the Åstrand Laboratory at GIH Sweden. The subject group was categorised as highly trained based on suggested criteria for competitive cyclist by Jeukendrup et. al.[114].

Before the experimental trials the subjects reported to the laboratory for a preliminary testing session in order to record personal characteristics, bike fitting and to determine VO_2max (Oxycon Pro, Erich Jaeger GmbH, Hoechberg, Germany) using an incremental test to fatigue. The VO_2max test included five four minute long periods at steady state to determine oxygen consumption at submaximal intensities at 100-300 Watt. After completing the submaximal part of the test and a short active rest of four minutes, the subjects started the incremental test

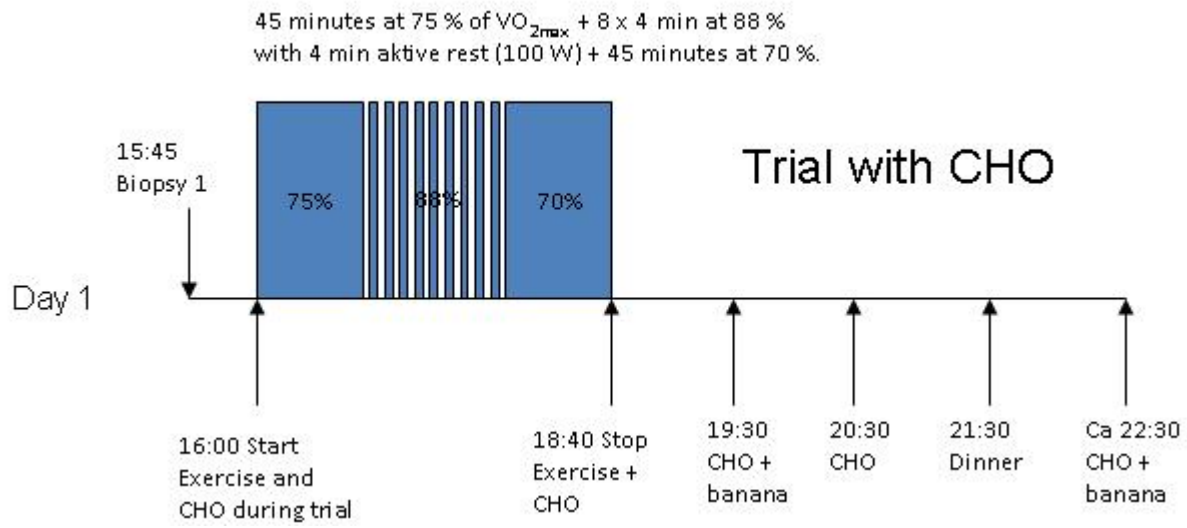
[114] AE Jeukendrup, NP Craig & JA Hawley, ”The bioenergetics of World Class Cycling”,J Sci Med Sport, 4(2000:3, Dec), p. 415.

to exhaustion in order to record VO_2max. The initial load for the incremental part of the test was based on the subject's rating of perceived exertion (Borg- category scale RPE) and respiratory exchange ratio (RER) during the last sub maximal ramp at 300 Watt and was calculated in order to elicit a maximal oxygen uptake within 7-8 minutes. All subjects started at 280-320 Watt and the load was increased with 20 Watt per minute until voluntary exhaustion. VO_2max was defined as the highest recorded oxygen uptake during 60 consecutive seconds. The VO_2max value was fitted to the linear relation of VO_2 and corresponding power outputs for the following exercises could thereafter be calculated. See appendix #5 for more details.

4.2 Experimental design

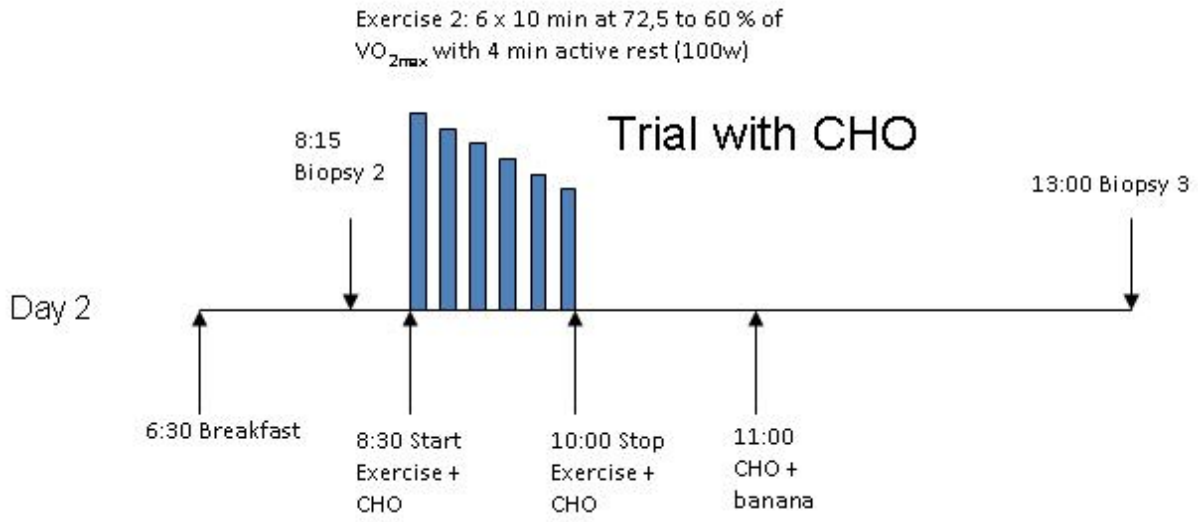
The experimental trials were performed in the spring and summer of 2010. The study was carried out in a crossover design and the subjects were told to rest the day before the trial during both conditions. Trials in CHO and non-CHO condition were separated by one or two weeks where training was conducted as usual for each subject. During the depletion exercise and exercise 2 the following day, water or a carbohydrate drink was supplied *ad libitum* during each subject's first trial. The subject then received the same amount of fluid during their second trials. Before the depletion exercise the subjects body weight was recorded and blood glucose and lactate was analyzed from a capillary sample collected from the fingertip (EKF-diagnostic GmbH, Germany). After calibration and adjustment of the bike according to the subject's personal settings as tested during the pre test, the exercises started with 45 minutes at 75% of VO_2max. Heart rate (Polar Electro Oy, Kempele, Finland) and Borg RPE was noted at same time intervals as capillary blood was sampled during the trial. After four minutes of rest the subjects then performed eight 4 minute long intervals at 88% of VO_2max with equal time of rest between intervals. The exercises then ended with an additional 45 minutes at 70 % of VO_2max. Blood glucose and lactate samples were collected at the end of the first 45 minutes, at the end of interval 2, 4, 6 and 8 and at the end of the last 45 minutes of exercise. During exercise 2 samples were collected at rest and during the final seconds of interval 2, 4 and 6. After completing the depletion protocol the subjects were weighed again in order to compare fluid intake to weight loss and then supplemented with either water or carbohydrates. During trials, the subjects were free to alter the cadence freely on the ergometer and thereby be able to select the cadence most individually proper to intensity and fatigue, hence optimising performance. See appendix #6 for more details.

Figure 1 – Timeline for the first day of trials in CHO state. In non-CHO trials no carbohydrates was provided during exercise and recovery.



The exercise 2 was performed after 13 hours of rest and after supplementation of either a CHO rich diet or a non-CHO diet. After register body weight and collecting resting blood glucose and lactate samples the subject entered the ergometer and performed an 8 minutes warm up session. The protocol then consisted of six 10 minute long intervals starting at 72.5% of VO_2max during the first interval and then declined with a load corresponding to 2.5 % of VO_2max during each interval so that the last interval was performed at 60% of VO_2max. Blood glucose and lactate was collected during the last seconds of interval 2, 4, and 6. Heart rate and BORG were frequently registered during exercise. In five subjects, oxygen uptake was measured during the latter three minutes of each interval. Venous blood sample was also collected in the same five subjects during the latter stage of interval 6 for analysis of free fatty acid content. After completing the exercise 2 the subjects rested quietly until biopsy sampling took place three hour post exercise. See appendix #7 for a more detailed schedule of exercise 2.

Figure 2 – Timeline for the second day of trials in CHO state. In non-CHO trials no carbohydrates was provided during exercise and recovery.



4.3 Nutritional design

All subjects registered their diet two days preceding the depletion exercise. They were instructed to ingest a balanced and normal diet and to avoid a very high intake of carbohydrates as often practised by endurance athletes before competitions and demanding exercises. They were also told to refrain from alcohol, tobacco and dietary supplements in the form of vitamins the three days preceding trial and caffeine during the day of trial. The subjects repeated their dietary intake for the second trial. During the CHO trial a glucose/maltodextrin drink was provided during the depletion exercise (Carbo 136, Dahlblads Sweden), during recovery immediately after exercise and during the evening. A solid meal was consumed as dinner. The following day after a solid breakfast, carbohydrates were also consumed during the exercise 2-trial, as recovery and during rest. In total, the amount of supplemented CHO was 12.6 $g \cdot kg^{-1}$ body weight during the whole CHO trial. During non-CHO trials no carbohydrates were provided as recovery during/after exercise or in the solid meals. The two meals consisted of egg, bacon and butter and the total amount of supplemented fat and protein corresponded to 1.6 $g \cdot kg^{-1}$ and 1.2 $g \cdot kg^{-1}$ of body weight. The diet in the different trials was thereby not isocaloric.

4.4 Biopsies

The subject was given local anaesthesia (2-3 ml Carbocain, 20 mg/ml, Astra-Zeneca, Södertälje, Sweden) and an incision in was made through the skin and fascia at one-third of

the distance between patella and anterior superior iliac spine. The biopsy was taken in vastus lateralis using a Bergström needle with the appliance of suction.[115] The biopsy samples were immediately frozen in liquid nitrogen and then stored at -80 ºC before being freeze-dried and analysed. Muscle biopsies were taken before the depletion exercise, after an overnight rest prior to exercise 2 and finally three hours post completion of exercise 2. In total each subject contributed with six biopsy samples. The biopsies were randomly collected so that biopsy 1 and 3 were from the same leg and biopsy 2 from the other leg (for each trial). The subjects that donated two biopsies from the right leg during their first trial thereby donated two biopsies from their left leg during their second trial.

4.5 Muscle glycogen analysis

The muscle glycogen content was analyzed using an enzymatic method based on a modified protocol previously described by Leighton et. al.[116]. Muscle biopsy samples were manually dissected and cleaned from blood and connective tissue under a microscope. Samples were divided into smaller pieces and then mashed and mixed to obtain the average characteristics of each sample. 1-2 mg of the homogenized samples were weighted and put into Eppendorf tubes before being dissolved in KOH and heated for 20 minutes at 70 °C. Blank and standards were prepared and further on treated as samples. After incubation, acetic acid was added to the samples and pH was adjusted to 4.8. Amyloglucosidase and NaAc were added and the samples were set to incubate for 2 hour at 40 °C. Hexokinas and glucose 6 phosphate dehydrogenase reagent solvent was added to the cuvettes and samples, standard and blank was added. Glucose content was determined at 340 nm wavelength using spectrophotometric analysis and expressed as glycosyl units mmol·kg dw^{-1}. See appendix 10 for glycogen analysis schedule.

4.6 Plasma free fatty acids and blood glucose

Venous blood samples were collected 15 to 30 minutes prior to each biopsy. Blood samples (3 ml) were centrifuged at 3000 g at 4°C for 5 minutes. Plasma was stored at -80 °C and later analyzed for the concentration of non-esterified fatty acids (NEFA) with a test kit based on an in vitro enzymatic colorimetric method (NEFA –HR (2) test kit, Wako Chemicals GmbH,

[115] J Bergström, "Percutaneous needle biopsy of skeletal muscle in physiological and clinical research", *Scandinavian Journal of Clinical and Laboratory Investigation*, 35(1975:7, Nov), p. 609-616.
[116] B Leighton, E Blomstrand, RA Challiss, FJ Lozemanm, M Parry-Billings, GD Dimitriadis & EA Newsholme, "Acute and chronic effects of strenuous exercise on glucose metabolism in isolated, incubated soleus muscle of exercise-trained rats", *Acta Physiologica Scandinavica*, 136(1989:2, Jun;), p. 179.

Neuss, Germany). Blood glucose was analyzed using an enzymatic method using spectrofotometric analysis. Capillary blood samples were collected from a fingertip and analyzed for lactate and glucose concentration using an automated analyzer (BIOSEN 5140, EKF Diagnostics, Barleben, Germany).

4.7 Statistics

Repeated measures ANOVA were performed using the statistical analysis program Statistica, version 9 (StatSoft Inc., Tulsa, Ok, USA) to detect differences of time (T), condition (C) or interaction between T and C. When significance effect of T and C appeared a post hoc test (Fischer LSD) was performed to access differences to base line values. Analyses of covariance (ANCOVA) were performed to detect differences between repeated measures with Borg RPE as dependent variable and glycogen content, blood glucose, heart rate and blood lactate as independent variables. Pearson product-moment correlation (Microsoft Corporation; Redmond WA, USA) was performed to access the degree of correlation between variables. The accepted level of significance was set to $p<0.05$. Data is generally presented as means ± SD if otherwise not stated.

5 Results

5.1 Evaluation of the protocol

The average power output in the non-CHO trial was 273±23 W during the depletion exercise session and 226±20 W during the exercise 2, corresponding to 74.3±3.1% and 63.6±4.3% of VO_2max respectively. Power output was similar in the CHO-trial (276±22 and 234±15 w respectively). If the given workload could not be maintained it was adjusted during the second trial to match the work of the first trial. However, two of the subjects who started with the CHO-trial, did not manage to complete the second exercise session at a corresponding load during the non-CHO trial and therefore did somewhat less work during their second trial.

5.2 Muscle glycogen, blood glucose and free fatty acids during rest

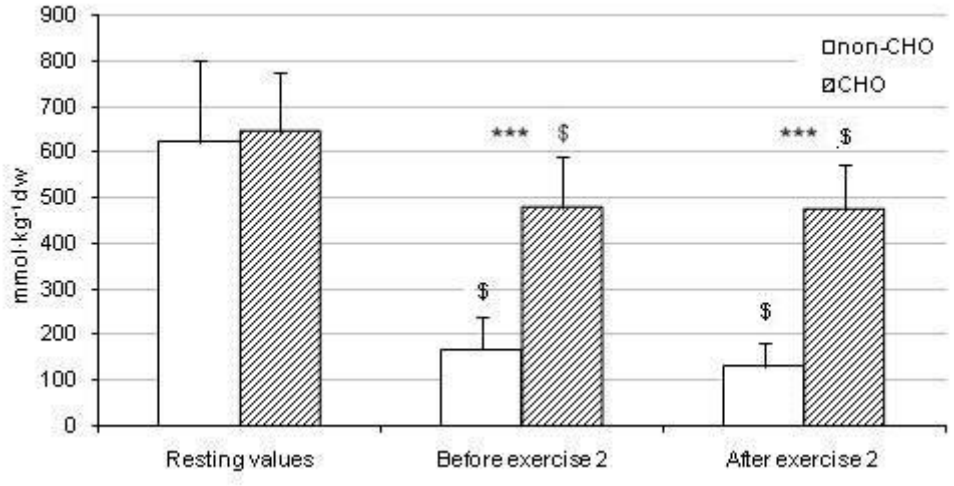


Figure 3 – Muscle glycogen content pre depletion exercise, in the following morning pre-exercise 2 and finally 3 hours post- exercise 2. Significant difference for repeated measures ANOVA was found between C and T ($p<0.001$). Significant difference between conditions * = $p<0.05$, *** = $p<0.001$, significant difference from resting values = $. Values as means ± SD (n=10).

Muscle glycogen content pre depletion exercise was 623±180 (range 446-1010) and 645±133 (range 449-852) mmol·kg dw^{-1} (glycosyl units) for non-CHO and CHO trials respectively. The depletion exercise followed by 13 hours of rest resulted in a significantly lower muscle glycogen content in the non-CHO trial ($p<0.001$), and the CHO trial ($p<0.001$) to 166±71 and 478±111 mmol·kg dw^{-1} respectively. After completion of exercise 2 and the following three hour long post exercise rest period there was no significant decrease in glycogen content although mean glycogen content in non-CHO trial was 130±52 mmol·kg dw^{-1} after exercise 2.

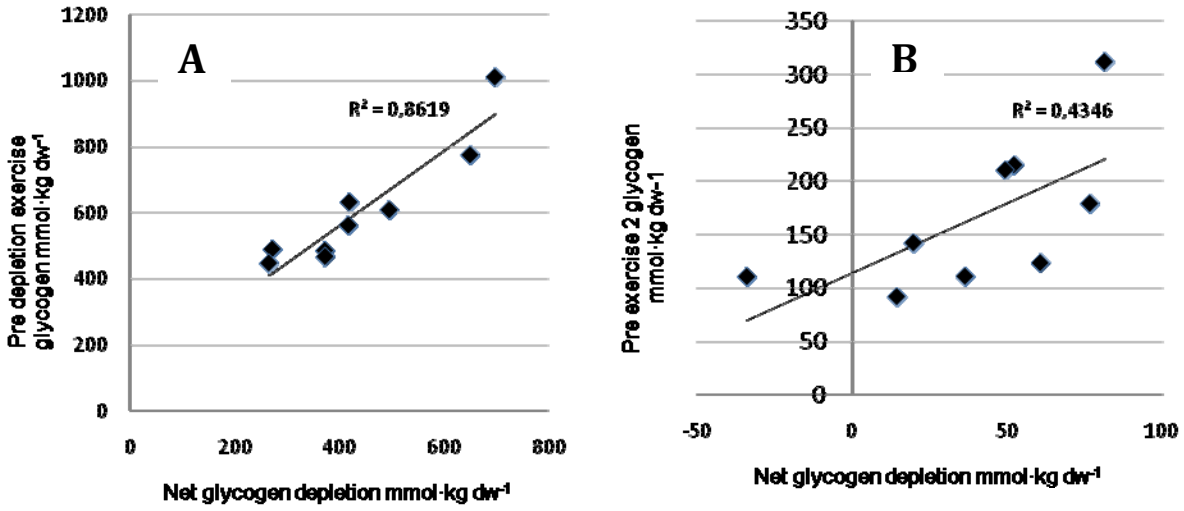


Figure 4– Correlation of individual glycogen storage in non-CHO state and net glycogen depletion after the depletion exercise (A) and after exercise 2 (B). Values as means ± SD (n=9).

In non-CHO state muscle glycogen content pre depletion exercise was positively correlated to the net glycogen depletion during the depletion exercise ($p<0.001$). Mean depleted amount of glycogen was 457 (range 267-698) mmol·kg dw^{-1} and glycogen content pre exercise 2 were almost correlated to the net depletion during exercise 2 ($p<0.054$). As visible in Figure 4 B one subject appears to have an increase in glycogen during exercise 2. This is highly unlikely and probably due to an unrepresentative biopsy sample.

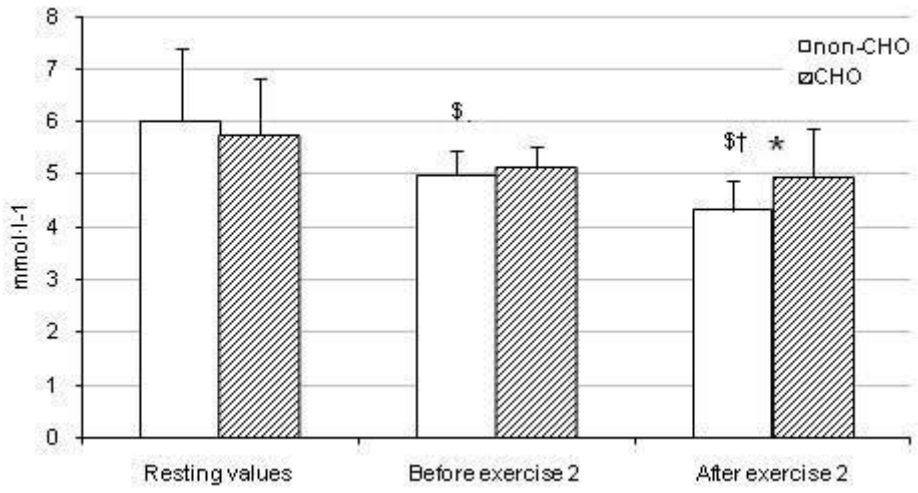


Figure 5 – Plasma glucose pre depletion exercise, in the following morning pre- exercise 2 and finally 3 hours post- exercise 2. Significant difference between conditions * = $p<0.05$, significant difference from resting values = $, significant difference as effect of time from previous value = †. Values as means ± SD (n=10).

There was no difference in resting plasma glucose concentration prior to depletion exercise in CHO compared to the non-CHO trial (6.01 and 5.75 mmol·L^{-1}, respectively). Repeated measures ANOVA displayed no further difference between conditions but there was a borderline significance ($p<0.068$) for C and T. Therefore, for exploratory reasons a post-hoc test for interaction effects was performed indicating differences for T and C. Resting blood glucose after the depletion exercise and following night was significantly lower in non-CHO trial (4.99 mmol·L^{-1} $p<0.01$) than pre values as well as after exercise 2 (4.30 mmol·L^{-1}, $p<0.001$) whereas in the CHO trial there was no significant alteration in blood glucose concentration during trial. After exercise 2 there was a significant difference between conditions.

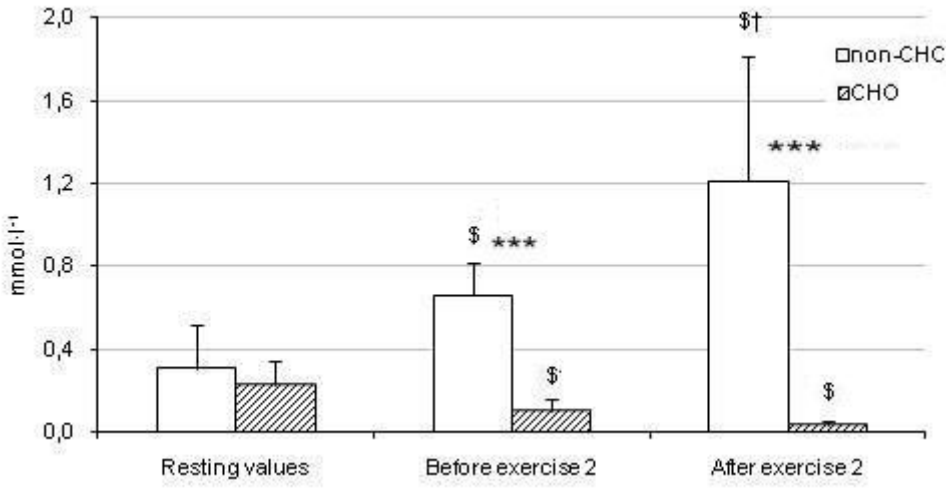


Figure 6 – Plasma FFA pre depletion exercise, in the following morning pre- exercise 2 and finally 3 hours post- exercise 2. Significant difference between conditions *** = $p<0.001$, significant difference from resting values = $, significant difference as effect of time from previous value = †. Values as means ± SD (n=10).

There was a marked difference in plasma FFA as a result of C and T. Resting plasma FFA values before both trials were similar at 0.31 and 0.23 mmol·L^{1} for the non-CHO and the CHO trial, respectively. During non-CHO trials values after depletion exercise were significantly elevated to 0.66 mmol·L^{-1} ($p<0.001$) and further increased to 1.21 mmol·L^{-1} ($p<0.05$) three hour after exercise 2. In CHO trial plasma FFA decreased to 0.10 mmol·L^{-1} ($p<0.05$) after depletion exercise and a nights rest and was further lowered to 0.03 mmol·L^{-1} after exercise 2, although not significantly different to pre exercise 2.

5.3 Physiological response during exercises

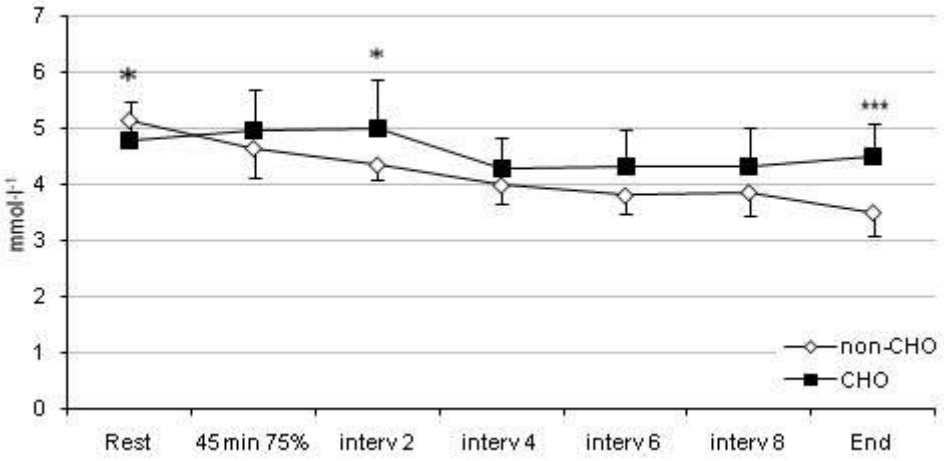


Figure 7 – Blood glucose concentrations during depletion exercise. Significant difference between conditions * = p<0.05, *** = p<0.001. No symbols for significance of T in the figure. Values as means ± SD (n=10).

Blood glucose significantly decreased as an interaction effect of C and T (p<0.001). A post-hoc test revealed significant differences between C in samples taken at rest, at interval 2 and at the end of exercise. Other physiological parameters (heart rate, blood lactate level or Borg RPE) that were measured during the depletion exercise displayed no significant difference between conditions although there was a trend in increased RPE during the latter stage of exercise for the non-CHO trial compared to CHO trial (see appendix 2 for figures)..

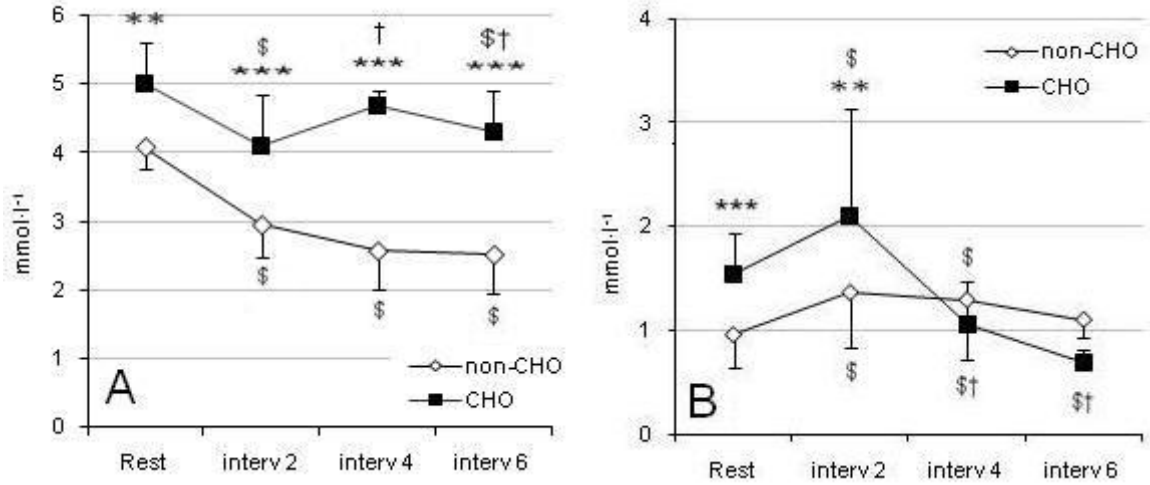


Figure 8 – Blood glucose (A) and lactate (B) concentrations during exercise 2. Significant difference between conditions * = p<0.05, ** = p<0.01, *** = p<0.001, significant

difference from resting values = $, significant difference as effect of time from previous value = †. Values as means ± SD (n=10).

In exercise 2 there was a significant interaction effect of C and T for blood glucose and lactate ($p<0.001$). In non-CHO trials blood glucose decreased during exercise and was lower in all intervals compared to resting values ($p<0.001$) whereas it remained stable in the CHO trial. Blood lactate concentration fluctuated in the CHO trial and was significantly different from non-CHO trials at rest and in interval 2 and 6.

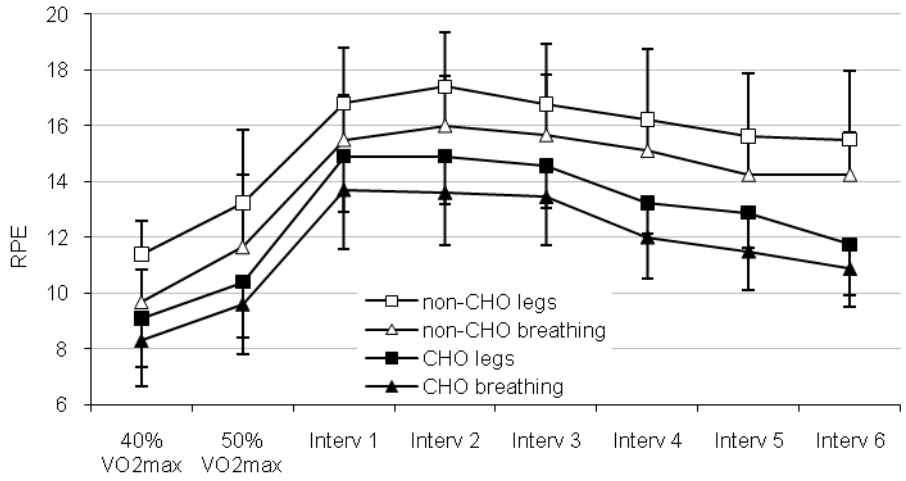


Figure 9 – Median Borg RPE of breathing and legs during exercise 2. No symbols of significance are displayed in the figure. Values as means ± SD (n=10).

There was a significant difference between conditions for RPE legs ($p<0.001$) and breathing ($p<0.01$) where RPE of both legs and breathing and were rated higher during a non-CHO state (non-CHO legs compared to CHO legs and vice versa). When controlled for markers of the individual's perceived exhaustion during non-CHO trials, analysis of covariance ANCOVA was performed with Borg RPE as dependent variable and with glycogen content, blood glucose, heart rate and blood lactate as independent variables. Significance appeared randomly and no single independent variable could be linked to Borg RPE-rating.

During exercise 2 breath gas exchange analysis was performed on the last five subjects to complete the study in order to collect further data possibly connected to the difference between training in low versus high CHO state. Values are presented in table 2 as a summarized means for each measured parameter during trials. No statistical analysis of variance was performed due to a small sample size.

Table 1 – Mean values for heart rate (HR), ventilation (VE), breath frequency (BF), Oxygen uptake (VO_2), carbon dioxide production (CO_2), respiratory exchange ratio (RER) and O_2/HR-quotient during exercise 2. Values as means collected during 3 minutes steady state at each interval and expressed as summarized means for each trial. % difference refers to relative change in non-CHO trial compared to CHO trial. (n=5).

	HR	V'E	BF	$V'O_2$	$V'CO_2$	$V'O_2 \cdot kg^{-1}$	RER	$O_2 \cdot HR^{-1}$
	bpm	$L \cdot min^{-1}$	$1 \cdot min^{-1}$	$mL \cdot min^{-1}$	$mL \cdot min^{-1}$	$mL \cdot min^{-1}$		mL/beat
non-CHO	155	92	49	3585	2838	48.8	0.79	23.6
CHO	143	85	39	3407	3086	45.8	0.90	24.2
Relative change in %	8.4	8.1	28.3	5.5	-7.9	6.8	-12.7	-2.3

6 Discussion

6.1 Muscle glycogen

The main purpose of the present study was to create and evaluate a method for glycogen storage manipulation. The protocol that was used resulted in a significant change in muscle glycogen content after the depletion exercise, measured after one nights rest and regardless of dietary supplementation. The glycogen content in non-CHO trial was however significantly lower than in CHO-trial. In CHO-trials, when the depletion exercise was combined with carbohydrate ingestion as well as a CHO-rich diet during 13 hour of recovery, muscle glycogen was restored to an average of ~74 % of pre depletion value compared to non-CHO trial where glycogen content were only ~27 % of pre values. If assumed that a similar magnitude in glycogen depletion occurred during both conditions, the mean resynthesis rate during the CHO-trial can be calculated to ~22 $mmol \cdot kg\ dw^{-1} \cdot h^{-1}$. The true synthesis rate however could be somewhat smaller due to a possible muscle glycogen sparing effect of CHO ingestion during exercise. Increased availability of exogenous CHO for oxidation suppresses plasma FFA and thereby reduces FFA oxidation and increase total CHO oxidation[117] but the elevated exogenous CHO availability also contribute to total CHO oxidation and thereby reduce the demand for glycogen utilization.[118] All supplemented CHO is not totally absorbed

[117] JF Horowitz, R Mora-Rodriguez, LO Byerley, EF Coyle, "Lipolytic suppression following carbohydrate ingestion limits fat oxidation during exercise", *American Journal of Physiology*, 273(1997:4 Pt 1, Oct), p. 771.
[118] J Bergström, p. 221f.

during exercise and the maintained blood glucose concentration and remaining unabsorbed CHO in the stomach, intestines and in blood will contribute to glycogen resynthesis immediately after exercise. In the present study, a supposed synthesis rate of ~22 mmol·kg $dw^{-1}·h^{-1}$ is high but with regard to the absolute magnitude of depletion and the high ingestion of carbohydrates of 6.9 $g·kg^{-1}$ during the 13 hours of recovery and the training status of subjects the rates are in order with previous publications. [119] In non-CHO trials net glycogen depletion correlated to pre depletion glycogen content ($p<0.001$) and the post exercise 2 glycogen content almost correlated to net depletion during exercise 2 ($p<0.054$). This was expected as previous research has shown that muscle glycogen depletion rates are dependent on stored glycogen availability[120]. According to the analysed biopsy sample from one subject, muscle glycogen increased from pre to post exercise 2 which is highly unlikely and most probably due to a unrepresentative muscle sample.

As mentioned earlier there is a resynthesis of muscle glycogen even when carbohydrates are not dietary supplemented. Rates of 7-18 mmol·kg $dw^{-1}·h^{-1}$ have been presented during the first four hours of recovery.[121, 122] Since muscular glucose uptake decline by time a assumed more modest mean rate of muscle glycogen synthesis during the full 13 h of recovery could be expected. The restored glycogen would partly be provided by gluconeogenesis. If assumed a mean resynthesis rate of ~4 mmol·kg $dw^{-1}·h^{-1}$, a subtraction of the theoretically resynthesized glycogen content gives mean glycogen content after depletion exercise to be calculated to 114±71 (range 40-252) mmol·kg dw^{-1}. This is just a theoretically estimated value but can possibly contribute to the discussion of the true effect of the actual glycogen depletion exercise since no post depletion exercise biopsies were taken.

In the present study we manage for the non-CHO trial to create a pre exercise 2 glycogen content corresponding to ~ 35 % of the glycogen content in CHO trial. Compared to previous research[123] [124] [125] [126] where pre exercise glycogen content in low CHO-trial of ~ 44 - 70 % (of corresponding value in high CHO trial) has been achieved, our result is to our knowledge

[119] R Jentjens, 2003, p. 117-44.
[120] HG Rauch, p. 35f.
[121] G van Hall, p. 1634.
[122] R Jentjens, 2003, p. P124.
[123] AK Hansen, p. 96.
[124] EG Churchley, p.1606
[125] WK Yeo (2009), p. 354.
[126] WK Yeo (2008), p. 1465.

the largest created difference in muscle glycogen content that has been achieved between conditions with the depleting exercise held equivalent between trials.

6.2 Plasma FFA and glucose

As an effect of exercise and the diet high in fat, free fatty acids measured in blood plasma was forcefully elevated during non-CHO condition during rest. Increased lipolysis is a direct consequence of a reduced plasma insulin concentration and increased cortisol, growth hormone and testosterone release and is expected when a non-CHO diet is practised after a training session. Although not measured in this study, exercise and low glycogen content can acutely increase catecholamine release thus stimulating fat metabolism and thereby partly explain the high plasma FFA-values. However, a long term effect of a low-CHO diet over two days following a depletion exercise does not seem to have an effect on catecholamine levels.[127] And as demonstrated by Mikulski et. al. in trained subjects a depletion exercise followed by a night of fasting resulted in a reduction in norepinephrine and insulin levels from pre values. When a high fat or a high CHO -breakfast was ingested after a night of fasting a reduction in insulin levels, decrease in RER and increase in growth hormone, testosterone, blood FFA content and unaltered catecholamine levels were observed in the high fat group in comparison to the high CHO group.[128] In the present study blood FFA was low during rest in CHO-trials. Pre exercise CHO ingestion suppresses plasma FFA content during exercise due to the insulin mediated inhibition of lipolysis.[129] The direct consequence of reduced blood FFA availability is a reduction in fat oxidation and CHO-ingestion pre/during exercise resulting in increased exogenous CHO availability also suppresses fat oxidation of intracellular triglycerides.[130]

Blood glucose during both conditions remained stable during rest after a night's recovery. But at 3-hours post exercise 2, blood glucose concentration was lowered and had not yet returned to a normal level from the very low values measured during non-CHO trials. This would be expected with regard to the individually low blood glucose values that were measured during

[127] AE Lima-Silva, R Bertuzzi, E Dalquano, M Nogueira, D Casarini, MA Kiss, C Ugrinowitsch & FO Pires, "Influence of high- and low-carbohydrate diet following glycogen-depleting exercise on heart rate variability and plasma catecholamines", *Applied Physiology, Nutrition, and Metabolism*, 35(2010:4, Aug), p. 545.
[128] T Mikulski, A Ziemba & K Nazar, "Metabolic and hormonal responses to body carbohydrate store depletion followed by high or low carbohydrate meal in sedentary and physically active subjects", *Journal of Physiology and Pharmacology,* 61(2010:2, Apr), p. 196ff.
[129] JF Horowitz, p. 771.
[130] E Coyle F, AE Jeukendrup, AJ Wagenmakers, WH Saris, "Fatty acid oxidation is directly regulated by carbohydrate metabolism during exercise",*American Journal of Physiology*, 273(1997:2 Pt 1 Aug), p. 271ff.

the latter stage of exercise 2 and the absence of carbohydrate supplementation during the following recovery. Whereas muscle glycogen storage alteration is a slower process, blood glucose and FFA concentrations is highly dependent on the supplemented diet. Ninety minutes before the blood sampling that was collected prior exercises 2, the subjects had in CHO trials ingested a breakfast high in carbohydrates or in non-CHO trial absent of carbohydrates but high in fat, which naturally has an affect on the presented values. CHO trials were further conducted with supplemented CHO during both exercises and during recovery which have an affect on values post exercise 2.

6.3 Completion of trials

As a summery, the performed amounts of work in the different trials were not significantly difference between conditions. The individual response to the selected workload was however varying. The depletion exercise protocol was manageable for three of the ten subjects. This was not due to a failure in the setting of the load and time of the protocol but merely a consequence of a protocol designed to be so hard and demanding as to ensure that glycogen levels would be effectively depleted. Eight of a total of ten subjects achieved identical performances during both CHO and non-CHO conditions during the depletion exercise. Five of the subjects requested a reduction in load during the last 45 minutes of steady state cycling at 70% of VO_2max, two of the subjects requested additional reduction of load earlier in the exercise and three subjects managed to sustain the load for the full time. Although there was no significant difference in performed work between trials, there was trend for reduced exercise capacity during the latter part of the depletion exercise in non-CHO trials. The influence of supplemented CHO on endurance ability between conditions was expected due to the energy contributing affect of CHO supplementation and not as a result of a placebo effect. This is strengthened by previous demonstrations by Hulston and Jeukendrup who demonstrated that a placebo-CHO induced increase in performance during endurance exercise is not likely to occur[131] and it is very well documented that carbohydrate supplementation during prolonged exercise has an effect on performance.[132]

Exercise 2 resulted in almost identical behaviour and individual performance for each subject as during the depletion exercise. The protocol was designed to correspond to a hard training

[131] CJ Hulston & AE Jeukendrup, "No placebo effect from carbohydrate intake during prolonged exercise", *International Journal of Sport Nutrition and Exercise Metabolism.* 19(2009:3, Jun), p. 283.
[132] AD Karelis, JW Smith, DH Passe, F Péronnet, "Carbohydrate administration and exercise performance: what are the potential mechanisms involved?", *Sports Medicine*, 40(2010:9, Sep), p. 747-763.

session and during the glycogen depleted state there was a marked difference in the individual response to fatigue. The protocol was adjusted after completion of trials for the first three subjects by reducing the initial intensity of exercise in an attempt to create a manageable exercise suitable for a randomisation of conditions. The exercise 2 protocol that consisted of 6·10 minutes started at ~80 % of VO_2max in the first edition and then reduced 5-2.5 % for each interval. However, it was hard to predict the magnitude of the individual response to the exercise intensity during the low glycogen state and after calculation and a evaluation of the results the protocol was adjusted so that the subjects performed the same amount of exercise of at total of 60 minutes at a average intensity of 65 % of VO_{2max} starting at 72.5 % and reduced by 2.5% after each interval. The same two subjects who experience difficulties in completing the depletion exercise in non-CHO trials failed to complete the performance exercise at a corresponding load during the two conditions. In summary the overall completions of protocols was successful.

6.4 Methodological limitations – exercising in low glycogen state

Rauch et. al. have suggested that a correlation exists between the individually lowest tolerable muscle glycogen content and the endurance pacing strategy adopted by the individual in order to complete an exercise within an individually predetermined critical muscle glycogen concentration.[133] This is suggested to be the result of a central feedback control since muscle activation plays a significant role in the regulation of glycolysis[134], hence governing the ATP demand in order to restrict the intensity of work to a sustainable level in accordance to critical muscle glycogen level. This is supported by the fact that the subjects in the present study experienced difficulties in sustaining the selected intensity of exercise during exercise 2 trial in the non-CHO state regardless of blood glucose and end glycogen content. We do not know the fibre-type specific glycogen depletion or if the development of fatigue can be dependent on the variability in fibre type composition between subjects. The present depletion and exercise 2 -protocol had a fixed duration and intensity individually calculated from the subjects' pretest and did not result in a maximal achievable depletion of glycogen storage nor was designed to push the subjects to exercise to an individual state of fatigue. With the exception of the two subjects that did not complete the exercises at the selected load and/or intensity. The individual setting of load was based on the subjects' oxygen consumption and required exercise load was calculated from oxygen requirement on sub maximal intensities of

[133] HG Rauch, p. 36.
[134] KE Conley, MJ Kushmerick & SA Jubrias, "Glycolysis is independent of oxygenation state in stimulated human skeletal muscle in vivo", *Journal of Physiology*, 511(1998:15, Sep), p. 944.

exercise and at VO_2max. Differences in individual lactate threshold and functional threshold power (the highest sustainable mean effect that can be tolerated during 60 minutes of exercise) may therefore influence the subjective perception of strain from the chosen work intensity and influence physiological parameters as well as the ability of the subject to perform the exercises. The consequence for the individual performances was that a few of the subjects may have been able to exercise at a higher load as well as the set load was too high for the subjects that fail to complete the exercises as calculated. For future research, functional threshold power may be a useful tool to estimate the work capacity at sub maximal intensities in individuals and thereby take into account the actual capacity of endurance performance on an individual basis.

The size of the individual glycogen storage may naturally influence exercise capacity and individual perception of fatigue during exercise 2 in depleted state. The subjects in the present study kept a diary of nutritional intake and replicated the same nutritional intake during their second trial. This resulted in small differences in individual glycogen content between trials but quite a large variation between subjects, yet there was no correlation between pre depletion exercise glycogen storage and the ability to complete the exercise 2 at pre set load. However, the effect of the amount of available glycogen cannot be ruled out based on trials in previous research performed under conditions similar to the present study. For example, the effect of higher glycogen levels on performance during normal training circumstances with the ingestion of breakfast 3 hours prior to exercise has been investigated. During 180 minutes of cycling exercise at 70% of VO_2max in well trained subjects Bosch et. al. observed a better maintenance of blood glucose and higher endogenous oxidation rates of glucose during the end of trial in the CHO-loaded group in comparison to the group that was supplied with a normal diet (initial muscle glycogen values of 830 versus 595 mmol·kg dw^{-1}). [135] The intensity and duration of the exercise was similar in energy expenditure as the depletion exercise in the present study as well as the individual pre glycogen storage. In Bosch et. al.'s study, there was a marked increase in FFA oxidation during the latter stage of exercise in the normal CHO-group and the authors concluded that fatigue was associated with reduced end glycogen content and not due to a decline in blood glucose. Interestingly, measured with radiolabeled tracers there was no glycogen sparing effect in the liver. [136] In a similar study by

[135] AN Bosch, SC Dennis & TD Noakes, "Influence of carbohydrate loading on fuel substrate turnover and oxidation during prolonged exercise", *Journal of Applied Physiology*, 74(1993:4, Apr), p. 1923ff.
[136] Ibid, p. 1923ff.

Burke et. al. where carbohydrates were supplied during exercise a carbohydrate loading protocol did not account for an improvement during a 100-kilometer time trial. It should be noted that the initial difference in resting glycogen and glycogen utilization values between groups were only 18 % and 11% higher in CHO-loaded group.[137] This indicates that blood glucose values could have remained high during both trials as an effect of the CHO-feeding, and that attainment of blood glucose might be a more important influencing factor than glycogen content at latter stage of exercise. In summary, the glycogen deposits prior to exercise and the ingested food prior to exercise can have influence on endurance capacity and end glycogen deposits in depleted state. This meaning that the individual differences in endurance capacity and end glycogen values is a result not merely of the hypothesized individual tolerance for depletion but also as a consequence of individual metabolic response to the chosen exercise intensity and pre exercise glycogen storage.

6.5 Exercise and fatigue

In this study some effort has been focused on collecting data concerning the development of fatigue and response to training in low glycogen state. Although there was an observed difference in physiological parameters RPE, blood glucose and lactate between conditions no single variable could be solely associated to increase in RPE. The development of fatigue appears to be complex and due to individual differences in training experience this might affect the individual tolerance to training in low glycogen state.

In studies investigating the effect of training with low muscle glycogen content a comparison to training in CHO-loaded state is inevitable. One critical difference between training in the different conditions is the ability to perform endurance training in low glycogen state since reduced ability to oxidize carbohydrates is associated with increased fatigue and thereby reduced performance during high intensity exercise[138]. The presumed increased oxygen demand by exercising in low glycogen state might partly be explained due to increased β-oxidation that elevates the oxygen cost for a fixed work rate. This should be accounted for when comparing conditions and the ability to perform endurance exercise. However, when calculated using the data in the present study, an increased β-oxidation does not correspond to

[137] LM Burke, JA Hawley, EJ Schabort, A St Clair Gibson, I Mujika & TD Noakes, "Carbohydrate loading failed to improve 100-km cycling performance in a placebo-controlled trial", *Journal of Applied Physiology*, 88(2000:4, Apr), p. 1286.
[138] CJ Hulston, MC Venables, CH Mann, C Martin, A Philp, K Baar & AE Jeukendrup, "Training with low muscle glycogen enhances fat metabolism in well-trained cyclists", *Medicine & Science in Sports & Exercise*, 42(2010:11), p. 2050.

the total sum of the increased oxygen demand in the non-CHO state. A difference in fibre recruitment and other factors might add to the explanation why exercising in a non-CHO state adds to metabolic strain. Better knowledge regarding the mechanisms controlling the development of fatigue might therefore be of value in the design of nutritional models as well as the design of exercise protocols and thereby increase the ability to standardize the intensity and duration of exercise during different conditions.

6.6 Conclusions

The muscle glycogen depletion protocol and dietary intervention used in the present study was successful in creating a difference in muscle glycogen storage. There was a positive correlation of pre glycogen content and the net glycogen depletion in non-CHO trial but no correlation between the definite pre content and depleted content were found. During exercise 2 in non-CHO trial Borg RPE, blood glucose and lactate were significantly different to the corresponding exercise performed with restored glycogen storage. Although individual low levels of glycogen content, blood glucose and differences in blood lactate are associated with fatigue and the individual rate of perceived exhaustion (RPE) could not be associated with one single dependent factor. The individual development of fatigue appears therefore to be dependent on several factors, all having an affect on performance and RPE.

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Appendix 1 – Search of literature

Aim and question

The aim of this study were to evaluate a method for muscle glycogen depletion as well as observing the resynthesis of muscle glycogen in subjects feed with either a carbohydrate rich diet or a diet completely in absence of carbohydrates. The aim was also to observe the response between conditions in physiological parameters such as blood glucose, blood lactate, heart rate, RER and BORG.

Vilka sökord har du använt?

Muscle glycogen
Depletion
Endurance
Exercise
GLUT-4
Carbohydrate
Glycogen synthesis, resynthesis
Carbohydrate loading

Var har du sökt?

Pubmed

Sökningar som gav relevant resultat

Pubmed: muscle glycogen and depletion and endurance 4 relevanta träffar Pubmed: muscle glycogen resynthesis 7 relevanta träffar

Kommentarer

Det mesta av det insamlade materialet kommer från genomläsning av referenslistor och ”related citations” på Pubmed. De flesta sökningar sker i flera steg och med specifika ordkombinationer utifrån informationsbehovet.

Appendix 2 – Fig 10-13

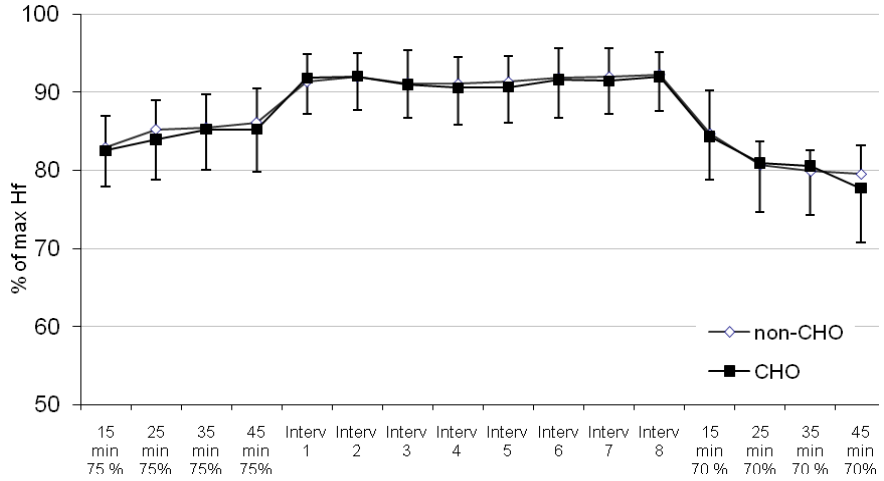


Figure 10 – Heart rate during depletion exercise. No significant difference between conditions (n=10).

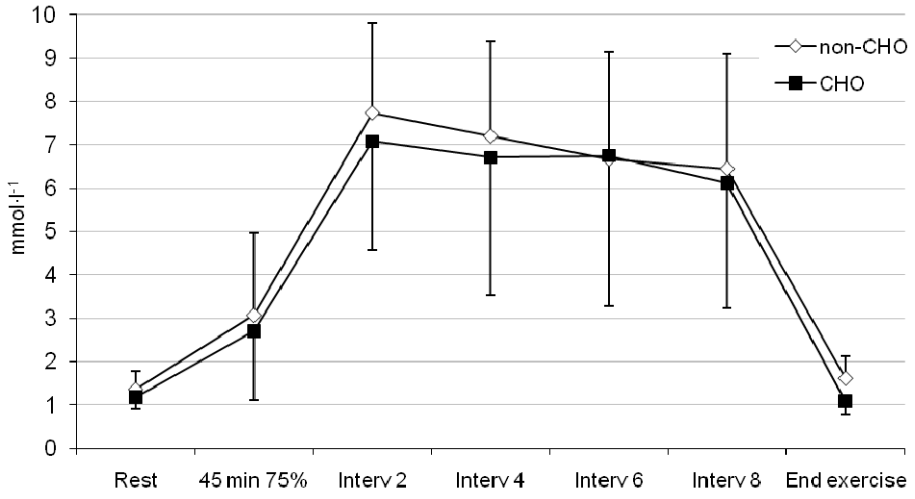


Figure11 – Blood lactate during depletion exercise. No significant difference between conditions (n=10).

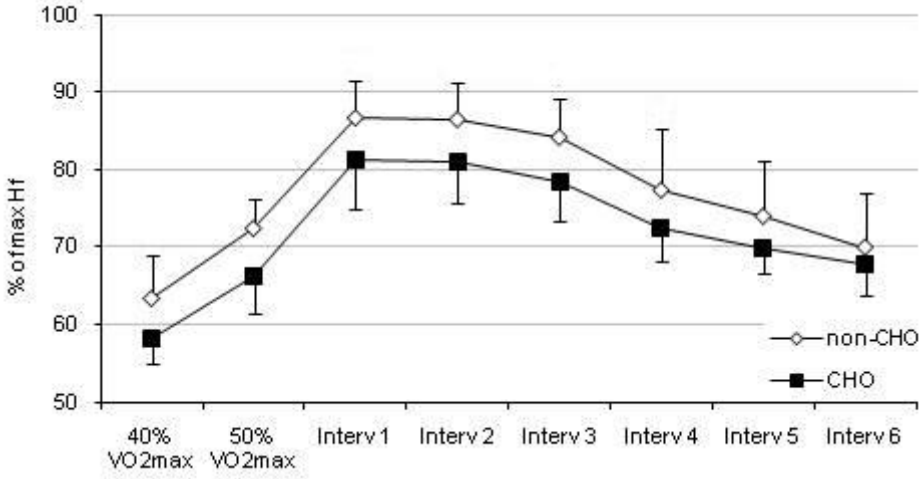


Figure 12 – Heart frequency during exercise 2. No significant difference between conditions (n=10).

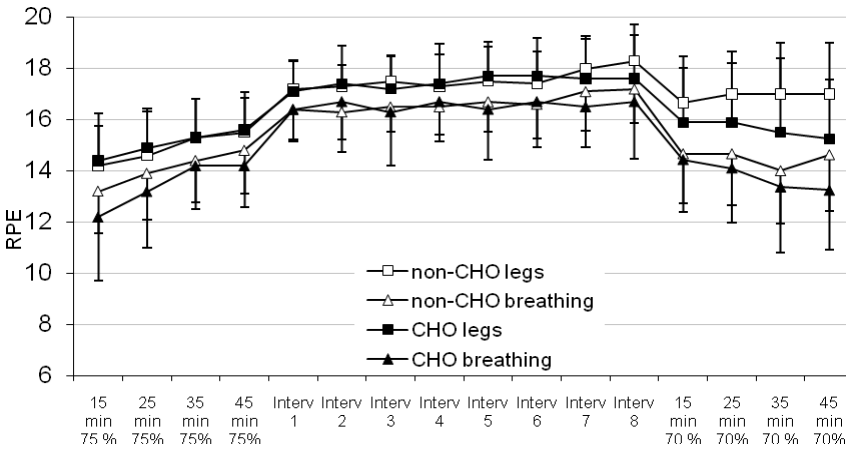


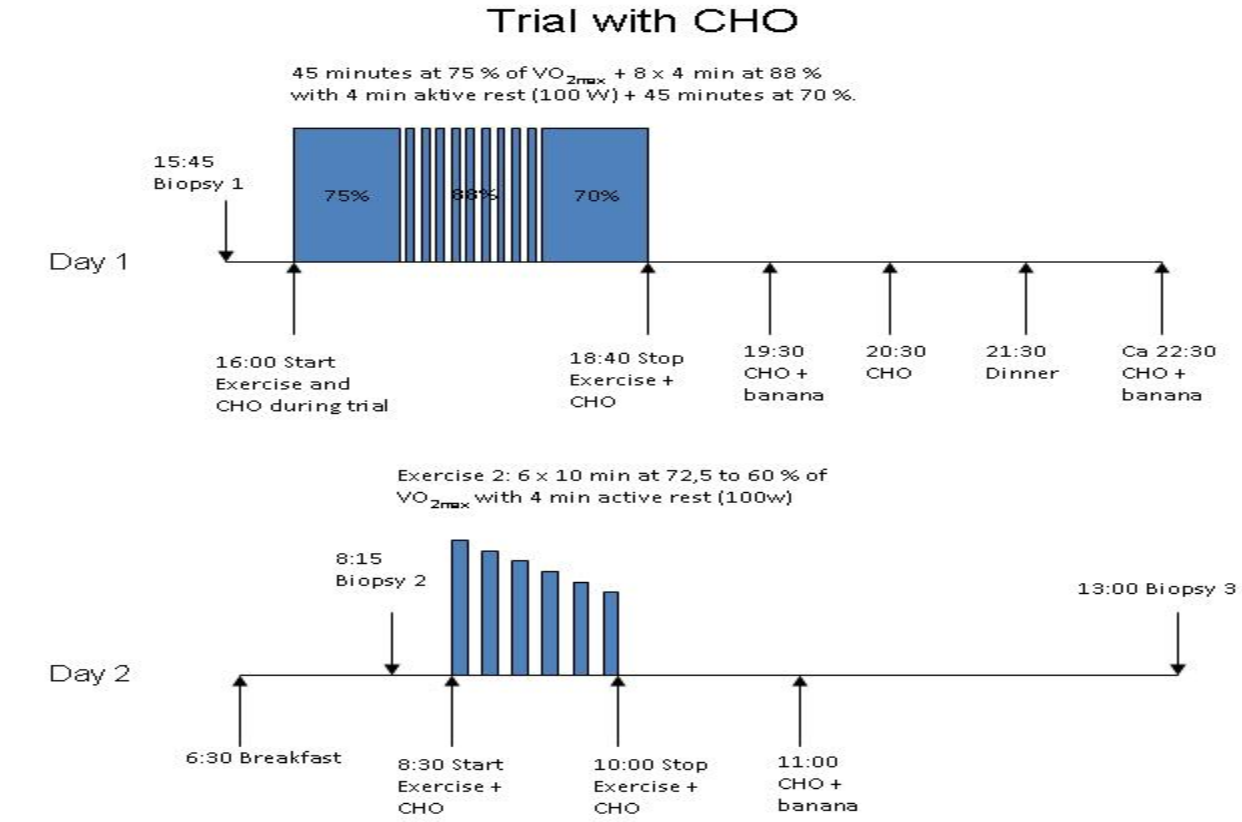
Figure 13 – Borg rating during depletion exercise. No significant difference between conditions although a trend towards increased RPE during the latter stage of exercise in non-CHO conditions (n=10).

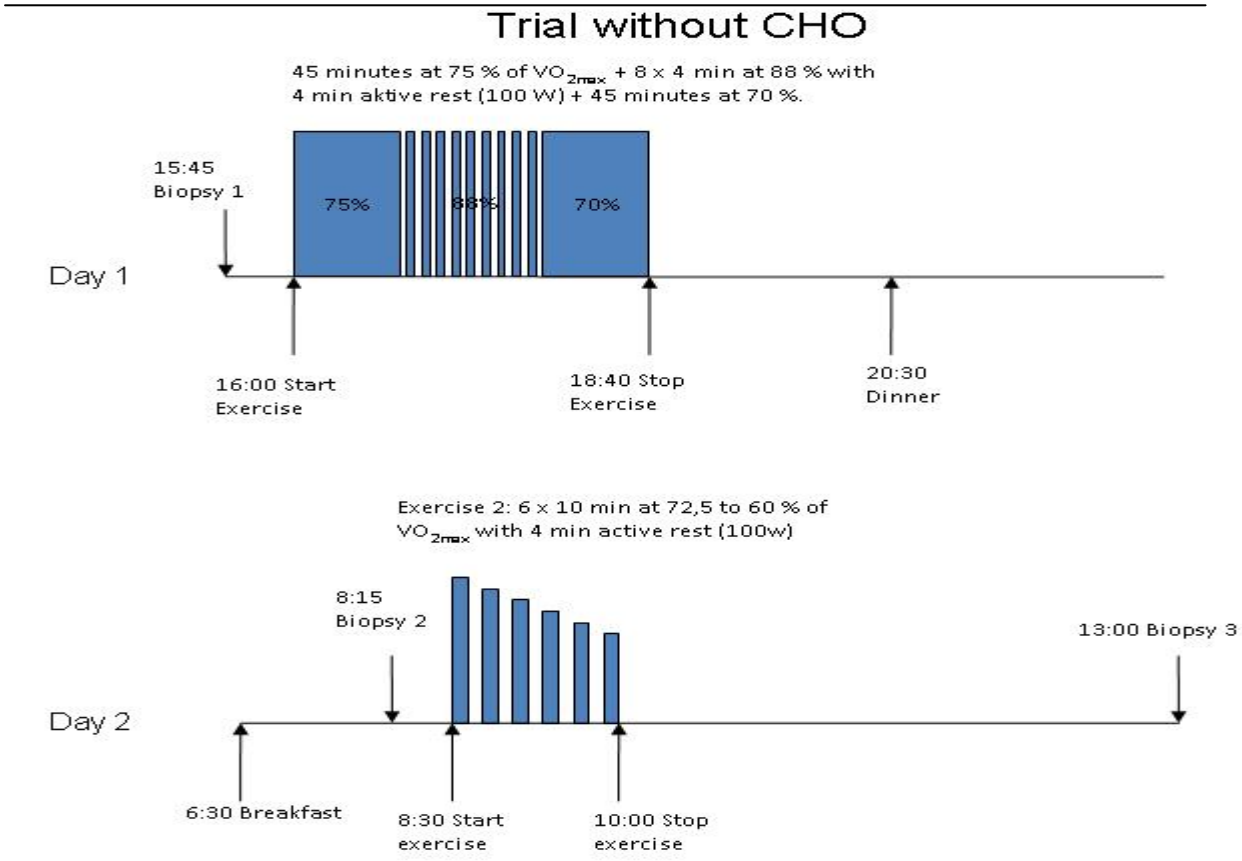
Appendix 3 – Full characteristics of subjects at pre-test

Values presented as means ± SD. Descriptive data from the VO_2max test is collected from the same minute as VO_2max was sampled.

Subject	Age [year]	BW [kg]	Hight [m]	BMI	Max Hla [mmol/L]	VO_2max [mL/min]	VO_2max [mL/kg-min]	Wmax	Max w/kg	Max HR [Bpm]	Max RER	Max V'E [L/m]
1	34	75.6	1.83	22.6	10.97	4921	65.1	400	5.29	193	1.17	187
2	27	68.5	1.80	21.1	18.01	4489	65.5	360	5.26	193	1.18	174
3	28	73.7	1.90	20.4	13.97	4995	67.8	423	5.74	175	1.24	191
4	33	79.2	1.87	22.6	13.33	4794	60.5	397	5.01	180	1.21	166
5	19	83.6	1.75	27.3	13.41	5339	63.9	375	4.49	206	1.15	192
6	30	70.7	1.80	21.8	14.49	4473	63.3	377	5.33	188	1.18	163
7	21	65.4	1.78	20.6	16.21	4468	68.3	340	5.20	198	1.26	173
8	32	84.5	1.88	23.9	16.64	5390	63.8	392	4.63	185	1.26	203
9	30	75.0	1.91	20.6	14.75	4923	65.6	398	5.31	187	1.23	188
10	24	71.0	1.78	22.4	13.94	4966	69.9	412	5.80	193	1.15	191
Mean	28	74.7	1.83	22.3	14.57	4876	65.4	387	5.21	190	1.20	183
SD	5	6.3	0.06	2.1	1.98	332	2.8	25	0.42	9	0.04	13

Appendix 4 – Timeline for trials





Appendix 5 – Protocol pretest

Protokoll Förtest

Test	Sadel h/sb	Pedaler:
Testperson	Sluttid test	
Personnummer	Vikt	
Datum 2010 Tid	Längd	

Period	Tid (min)	w	RPM	VO2 (ml)	HF	Borg andning	Borg ben	Hla	Blod Glukos
								*	
Steady state	0-4	100							
Steady state (4min)	0-4	100							
Steady state	4-8	150							
Steady state (4min)	4-8	150							
Steady state	8-12	200							
Steady state (4min)	8-12	200							
Steady state	12-16	250							
Steady state (4min)	12-16	250							
Steady state	16-20	300							
Steady state (4min)	16-20	300						*	
Rest (2 min)	20-22	100							
Rest (2 min)	22-24	200							
Nivå 1 (60sek)	24-25								
Nivå 2 (60sek)	25-26								
Nivå 3 (60sek)	26-27								
Nivå 4 (60sek)	27-28								
Nivå 5 (60sek)	28-29								
Nivå 6 (60sek)	29-30								
Nivå 7 (60sek)	30-31								
Nivå 8 (60sek)	31-32								
Nivå 9 (60sek)	32-33								
Nivå 10 (60sek)	33-34								

Ifyllt informationsbrev ☐

Ifyllt hälsodeklaration ☐

Hla slut test	*	
Hla post test 3 min	*	

Appendix 6 – Protocol depletion exercise

Protokoll tömningspass

Test		Sadel h/sb	
Testperson		Pedaler:	
Personnummer		Vikt före/efter	
Datum	Tid	Längd	
		Totalt vätska intaget under test	

Tid (min)	% VO_2max	watt	RPM	HF	Borg andning	Borg ben	Hla	Glu	Vätska?	Fläkt	Temp
Vila							*				
0-15	75%										
15-25	75%										
25-35	75%										
35-45	75%						*				
45-48		100									
48-52	88%										
52-56		100									
56-1:00	88%						*				
1:00-1:04		100									
1:04-1:08	88%										
1:08-1:12		100									
1:12-1:16	88%						*				
1:16-1:20		100									
1:20-1:24	88%										
1:24-1:28		100									
1:28-1:32	88%						*				
1:32-1:36		100									
1:36-1:40	88%										
1:40-1:44		100									
1:44-1:48	88%						*				
1:48-1:52		100									
1:52-2:07	70%										
2:07-2:17	70%										
2:17-2:27	70%										
2:27-2:37	70%						*				

Appendix 7 – Protocol exercise 2

Protokoll pass 2

Test		Sadel h/sb	
Testperson		Pedaler:	
Personnummer		Vikt före/efter	
Datum	Tid	Längd	
		Totalt vätska intaget under test	

Tid (min)	% VO_2max	watt	RPM	HF	Borg andning	Borg ben	Hla	Glu	Vätska	Fläkt	Temp
Vila							*				
0-6	40%										
6-8	50%										
8-18	72,5%										
18-22	40%										
22-32	70,0%						*				
32-36	40%										
36-46	67,5%										
46-50	40%										
50-60	65,0%						*				
1:00-1:04	40%										
1:04-1:14	62,5%										
1:14-1:18	40%										
1:18-1:28	60,0%						*				

Appendix 8 – Health formular

Hälsokontroll - Personupplysningar

Datum _____

Namn _____ Personnummer _____

Adress _____ Postnr_____

Telefon _____ E-mail _____ _____

Mobil_____

Har du eller har du tidigare haft någon av följande sjukdomar?

		Nej	Ja
1.	Ofta förekommande förkylningar	☐	☐
	Kraftig förkylningar	☐	☐
	Senaste behandling med antibiotika _____		
2.	Halsfluss eller annan halsinfektion	☐	☐
3.	Hösnuva	☐	☐
4.	Nässelfeber	☐	☐
5.	Astma	☐	☐
6.	Överkänslighet för föda, medicin, tvättmedel el dylikt?	☐	☐
7.	Har du fått bedövning hos tandläkare?	☐	☐
	Har du fått någon reaktion på detta?	☐	☐
	Om Ja vilken _____		
8.	Diabetes (sockersjuka)	☐	☐
9.	Lungjukdomar	☐	☐
10.	Hjärtsjukdomar	☐	☐
11.	Högt blodtryck	☐	☐
	Lågt blodtryck	☐	☐

12. Magsår, mag- eller tarmkatarr ☐ ☐
Medicin mot magbesvär ☐
☐
13. Någon form av gulsot ☐
☐
14. Leversjukdomar? ☐
☐
15. Ryggbesvär? ☐
☐
16. Huvudvärk (ofta förekommande eller migrän) ☐ ☐
Äter du smärtstillande medel mot huvudvärk? ☐ ☐

17. Använder du sömnmedel? ☐
☐
18. Har du någon gång haft sjukdom eller skada som krävt sjukhusvård? ☐ ☐
När, var, för vad……
19. Har du ordinerats medicin för långtidsbruk? ☐ ☐
Vilket läkemedel? När?

20. Tar du medicinen fortfarande? ☐
☐

Övriga upplysningar

20. Tidigare idrottsaktiviteter/träning ☐ ☐

21. Nuvarande idrott/träning ☐
☐
Vad och hur ofta _____

22. Röker eller snusar du ☐ ☐
Hur mycket.....

22. Äter du all sorts mat? ☐
☐
Om inte precisera.....

23. Äter du någon form av kosttillskott (vitaminer, proteiner etc) ☐ ☐

24. Känner du dig fullt frisk? ☐
☐

Appendix 9 – Letter of consent

Information till försökspersoner (reviderad 2010).

Projekttitel: Effekt av träning och nutrition på muskelns fettförbränningskapacitet.
Ansvariga: Forskningshuvudman: Gymnastik- och Idrottshögskolan.
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Bakgrund/syfte: Förbränningen av fett och kolhydrater sker i en del av cellen som heter mitokondrier. Muskulaturens kapacitet till till fettförbränning är beroende av förbränningsmotorns storlek (mängd mitokondrier), men påverkas även av andra faktorer. Syftet med den här studien är att jämföra effekten av en fettrik och kolhydratrik kost på fettförbränningen (studie 2). Resultaten från det här projektet förväntas ge bättre kunskap om hur träningen skall optimeras samt att ge en ökad kunskap om hur fettförbränningen regleras.

Hur går studien till? Vid ett separat förförsök bestäms din maximala syreupptagningsförnåga (VO_2max) vid cykling, genom att analysera volym och gassammansättningen på utandningsluften.

I studie 2 kommer vi att undersöka muskelns aeroba anpassning och muskelns förmåga till fettförbränning efter olika typer av kost. Efter ett inledande arbetspass där muskelns kolhydratförråd töms kommer du antingen äta en fettrik kost (FK) eller en kolhydratrik kost (KK), (randomiserad ordning och minst 2 veckor mellan försöken). Dagen efter tömningspasset gör du ett nytt standardiserat arbete. Muskelprov från lårmuskeln (se nedan) kommer att tas före det första arbetspasset och 3 timmar efter det sista arbetspasset (sammanlagt antal muskelprov 4st). Blodprov kommer att tas från en blodåder (ven) i armvecket (sammanlagd blodmängd <20ml). Total tidsåtgång för studie 2 uppskattas till ca 23 timmar uppdelat på tre försökstillfällen (inklusive förförsök).

Muskelbiopsi – risker, obehag: Muskelbiopsi innebär att en liten bit muskelvävnad (0,1-0,15 gram) tas ut med en specialnål. Muskelbiopsi utförs efter lokalbedövning av huden och underliggande bindväv. Ett 4-5 mm långt snitt görs genom huden, genom vilket biopsinålen förs in och ett muskelprov tas ut. Själva ingreppet med biopsinålen är över på ett par sekunder. I allmänhet känns en muskelbiopsi som ett trubbigt slag mot benet. I vissa fall kan en skarpare smärta kännas, som går över så fort nålen tas ut. Någon gång händer det att en blodutgjutning sker i muskeln, vilket kan innbära svullnad och värk under några dagar upp till en vecka. För att förhindra blodutgjutning lägger vi ett lokalt tryckförband över biopsistället, som skall vara kvar under 1-2 timmar. Liksom vid alla hudsnitt kan en hudnerv skäras av med lokalt känselbortfall som följd. Vid den här typen av biopsi är denna komplikation mycket ovanlig. I de fåtal fall där denna komplikation har ägt rum har allt normaliserats efter 6-12 månader.

Skötselinstruktioner vid muskelbiopsi: Under veckan före muskelprovtagning får du ej använda magnecyl eller någon annan medicin som innehåller acetylsalicylsyra (alvedon går bra). Dygnet före testerna får du ej utföra något tungt fysiskt arbete (>30 min) eller använda alkohol. Under veckan efter undersökningen skall du inte bada (p.g.a. infektionsrisk) och när du duschar skall du skydda området över biopsistället med plast. De inre vita långsmala plåstren skall du inte byta själv – de ramlar av efter ca 1 vecka.

Hantering av data/sekretess: Proverna och undersökningsresultaten kommer att kodas. Endast försöksledare och medarbetare kan koppla provresultaten till namn. Dina resultat kommer att behandlas så att inte obehöriga kan ta del av dem. Efter studiens avslutande kan du kontakta försöksledaren och få möjlighet att ta del av dina resultat.

Försäkring/ersättning: Patientskadeförsäkringen gäller för undersökningen. För deltagande i försöket utgår ersättning med 2500 Kr för studie 2.

Kontakta försöksledaren (Kent Sahlin) vid behov av ytterligare information.

Jag har muntligen informerats och har fått tillfälle att ställa frågor. Jag har tagit del av ovanstående skriftliga information och samtycker till deltagande i studien. Jag är medveten om att mitt deltagande är helt frivilligt och att jag när som helst och utan närmare förklaringar kan avbryta mitt deltagande.

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datum Namnteckning

Appendix 10 – Glycogen analysis schedule

Glykogenanalys Psilander/Flockhart 2010-07-12

1. Väg prover. Mellan 1-3 mg är okay
2. Sätt proverna i eppendorfrör och spinn dem 30 s på 7600 rcf
3. Märk rör för prover, blank och glykogen från kanin
4. Tillsätt 150 µl KOH till prover + blank
5. Vortexa och spinn proverna 5 s för att få bort fibrer från väggarna, vortexa ytterligare för att undvika bottensats
6. Sätt proverna i värmeblock 20 min eller i vattenbad 15 min på 70ºC
7. Ta ut proverna och sätt på is. Sätt ny temperatur på 40ºC i värmeblocket
8. Blanda lösningarna:
   - 3000 µl NaAc + 100 µl acetic acid
   - 3750 µl NaAc + 5 mg Amyloglukosidase
9. Ta proverna från is, vortexa och spinn sedan 5s på 7600 rcf. Vortexa igen för att undvika pellet
10. För över 75µl av prover, glykogen och blank till nya uppmärkta rör
11. Tillsätt **155** µl NaAc + Hcl och **150**µl NaAc + Amyloglukosidase till proverna, i den ordningen
12. Vortexa och sätt proverna i vattenbad 40ºC i 2h
13. Blanda reagenslösning:

	För 15-16 prov	**För 6-7 prov**
ATP	82 mg	41 mg
NADP	10 mg	5 mg
TEA-buffer	13 ml	6,5 ml
Dest H2O	6,96 ml	3,48 ml
G-6-dh	20 µl	10 µl
Hk	20 µl	10 µl

14. Vortexa reagenslösningen och ta fram kyvetter som märks
15. Tillsätt 500 µl reagenslösning till kyvetter för prov och blank. 540 µl till glukosstandard
16. Ta prover ur vattenbad och vortexa, spinn sedan på 60 s på 12 000 rcf
17. Blanda 50 µl prov i kyvetter och vortexa på låg hastighet
18. Låt stå 20 min och läs sedan på 340 nM på fotrospektrometern

Beräkna glykogenkoncentrationen:

$$\frac{(\text{abs.prov} - \text{abs.blank}) \bullet \text{uL totalt i inkuberad lösning} \bullet \text{uL totalt kyvettinehåll} \bullet \text{uL KOH})}{\text{vikt mg muskel} \bullet \text{uL prov i inkuberad lösning} \bullet \text{uL prov i kyvett} \bullet \text{NADH-faktorn (6.22)}} = \text{glycosyl units mmol x kg dw-1}$$