A comparison of calf muscle glycogen synthesis, measured by 13C MRS, and whole body muscle glycogen synthesis, measured by isotope methods, points to non-equal muscle glycogen synthesis rates

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Abstract

Glycogen synthesis rates were determined for whole body muscle by an isotope assessment and locally for the calf muscle (gastrocnemius) by ^{13}C MRS using ^{13}C-1-glucose infusion. The results indicate that the calf muscle glycogen synthesis rate may not account for the average whole body muscle glycogen synthesis rate, which likely is caused by differences in energy metabolism of various muscles or training conditions.

Introduction

Reduced glucose uptake and glycogen synthesis in muscle are hallmarks of insuline resistance. Glucose uptake is commonly assessed for the whole body during an euglycemic/hyperinsulinemic clamp, from which non-oxidative glucose uptake may be derived, using a separate measurement, as a reflection of whole body glycogen synthesis. Previously, glycogen synthesis as assessed by ^{13}C MRS of human calf muscle, using infusion with ^{13}C-1-glucose during an hyperglycemic or euglycemic and hyperinsulinemic clamp appeared to account for about 90% of whole body muscle glycogen synthesis as assessed by indirect calorimetry [1,2]. However, the indirect calorimetric method does not properly discriminate between glycolysis and glycogen synthesis and a more precise measure is possible using isotope methods [3]. In order to assess the correspondence between whole body muscle glycogen synthesis and local glycogen synthesis in calf muscle we examined volunteers twice; 1) by the isotope method [3] and 2) by ^{13}C MRS of the calf muscle using ^{13}C-1-glucose [4].

Methods

Six young lean subjects participated in this study (Age 22.8 yr ± 2.1; BMI 22.3 ± 0.5). 1) Whole body muscle glycogen synthesis and glycolytic rates were determined by isotope techniques [3] following the next protocol. After an overnight fast (14 h) the subjects were studied in the supine position with a catheter inserted into an antecubital vein of each arm. One catheter was used to sample arterialized blood with use of a heated hand box (60°C). The other catheter was used to infuse [6,6-$^2H_2$]glucose, [3 ^{3}H]glucose, a 20%-glucose solution and insulin. After a blood sample was taken to measure the background enrichment and specific activity of plasma glucose, a primed, continuous infusion of [6,6-$^2H_2$]glucose (>99% enriched; Cambridge Isotope Laboratories, Cambridge, MA) at a rate of 0.22 µmol•kg^{-1}•min^{-1} (prime: 17.6 µmol/kg) and a primed, continuous infusion of [3 ^{3}H]glucose (74kBq/ml) at a rate of 0.0032 µCi•kg^{-1}•min^{-1} (prime: 0.4 µCi•kg) was started. At T=0 insulin (100 kU Actrapid/L; Novo Nordisk Farma BV, Zoeterwoude, Netherlands) was started at a rate of 60 mU•m body surface area^{-2}•min^{-1}. After 60 minutes blood samples were drawn during 2 hours at a 10-minutes interval for measurement of specific activity of plasma glucose and H_2O for calculation of glycolysis [3]. Between 160 and 200 min a blood sample was drawn every 10 minutes for measurement of isotopic enrichment for calculation of rate of disposal of glucose. Plasma glucose concentrations were measured every 5 min with a Glucose Analyzer 2 (Beckman, Palo Alto, CA) and the 20%-glucose solution was infused at a variable rate to maintain euglycemia at 5.0 mmol/L. [6,6-$^2H_2$]Glucose and [3 ^{3}H]glucose was added to the infusate containing the 20%-glucose solution to prevent negative Rd artifacts during hyperinsulinemia. After 60 and 180 minutes blood samples were drawn for measurement of insulin, C-peptide, glucagon, cortisol and catecholamines. Body composition was determined by a DEXA-scan.

2) ^{13}C MRS measurements were performed on a separate day applying the same euglycemic hyperinsulinemic clamp but infusing 20% glucose, 30% enriched with 1-^{13}C-glucose. During the measurements, the subjects were lying inside a 1.5 T magnet (Vision, Siemens Erlangen) with the gastrocnemius muscle of the right leg positioned on top of a concentric ^{13}C surface coil. For ^{1}H acquisition, decoupling, and shimming a quadrature coil was used. ^{13}C MR spectra were obtained using an adiabatic pulse and a repetition time of 180 ms (2500 scans; 15 min blocks). During the first 60 ms of acquisition continuous wave decoupling (26 W) was applied. Glycogen and glucose C1 signals and the creatine C4 signal were evaluated and glycogen synthesis rate was determined as the increase in glycogen signal in time, corrected for plasma 1-^{13}C-glucose enrichment level.

Results:

Whole body glucose uptake during the isotope assessment and ^{13}C MRS protocols were the same (55±17 and 52±17 µmol/kg/min respectively) validating the physiological similarities of the protocols in the two assessments. The isotope method revealed a glycolytic rate of 16 ± 6 µmol/kg/min and a glycogen synthesis rate of 115 ± 26 µmol/kg/min. In contrast the glycogen synthesis rate as assessed by ^{13}C MRS of calf muscle was significantly higher (178 ± 72 µmol/kg/min). Glycogen synthesis expressed as percentage of whole body skeletal muscle glucose uptake was 69 (range 59 – 78 %) as derived from the isotope assessment, and 108 (range 52 – 155 %) as derived from the ^{13}C MRS measurements.

Discussion:

Differences in human muscle glycogen synthesis rate between the whole body assessment with isotopes and the local ^{13}C MRS measurements can only be understood when the glycogen synthesis rate is different between different skeletal muscles. The data indicates that in a number of subjects the gastrocnemius shows a higher glycogen synthesis rate than the average skeletal muscle. We also note that the variability of the whole body muscle glycogen synthesis rate is far less than that of the gastrocnemius muscle. These results may reflect different energy metabolism in various muscle possibly related to fiber type distribution which may also vary for the gastrocnemius as the result of training. The present results are in agreement with findings that exercise enhanced glycogen synthesis in calf muscle better correlates with basal glycogen synthesis in this muscle than with whole body glucose uptake [5]

References

[1] Shulman G.I. et al, N. Engl. J. Med. **322** (4): 223-228,1990
[2] Rothman D.L. et al, Proc Natl Acad Sci USA **92**: 983-987, 1995
[3] Rossetti L. et al, Am J Physiol **265**: E761-E769, 1993
[4] van den Bergh AJ et al, Eur J.Clin Invest **30** (2): 122-8, 2000
[5] de Haan C et al, ISMRM workshop on Dynamic Spectroscopy, Orlando, 2003