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Impaired repletion of intramyocellular lipids in patients with growth hormone deficiency after a bout of aerobic exercise

Hannah Loher[a,b,1], Stefan Jenni[a,c,1], Julie Bucher[a,c], Marion Krüsi[a], Roland Kreis[d], Chris Boesch[d], Emanuel Christ[a,e,*]

[a] Division of Diabetology, Endocrinology, Clinical Nutrition and Metabolism, Inselspital, Bern University Hospital, University of Bern, Switzerland
[b] Department of Medicine, Cantonal Hospital St. Gallen, St. Gallen, Switzerland
[c] EndoDia Praxis, Biel, Switzerland
[d] Department of Biomedical Research & Institute of Interventional, Diagnostic and Pediatric Radiology, University of Bern, Bern, Switzerland
[e] Division of Endocrinology, Diabetology and Metabolism, University Hospital of Basel, Basel, Switzerland

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ABSTRACT

Background: Ectopic lipids such as intramyocellular lipids (IMCL) are depleted by exercise and repleted by diet, whereas intrahepatocellular lipids (IHCL) are increased immediately after exercise. So far, it is unclear how ectopic lipids behave 24 h after exercise and whether the lack of growth hormone (GH) significantly affects ectopic lipids 24 h after exercise.

Methods: Seven male patients with growth hormone deficiency (GHD) and seven sedentary male control subjects (CS) were included. VO_{2max} was assessed by spiroergometry; visceral and subcutaneous fat by whole body MRI. ^{1}H-MR-spectroscopy was performed in M. vastus intermedius and in the liver before and after 2 h of exercise at 50% VO_{2max} and 24 h thereafter, while diet and physical activity were standardized.

Results: Sedentary male subjects (7 GHD, 7 CS) were recruited. Age, BMI, waist circumference, visceral and subcutaneous fat mass was not significantly different between GHD and CS. VO_{2max} was significantly lower in GHD vs. CS. IMCL were diminished through aerobic exercise in both groups: (−11.5 ± 21.9% in CS; −8.9% ± 19.1% in GHD) and restored after 24 h in CS (−5.5 ± 26.6% compared to baseline) but not in GHD (−17.9 ± 15.3%). IHCL increased immediately after exercise and decreased to baseline within 24 h.

Conclusion: These findings suggest that GHD may affect repletion of IMCL 24 h after aerobic exercise.

1. Introduction

The energy required for physical exercise is supplied by augmented availability and oxidation of glucose and free fatty acids (FFA) [1]. These metabolic adaptations are regulated by hormones such as cortisol, catecholamines and growth hormone (GH) [2]. In particular, GH is an important anabolic hormone after physical exercise. Specifically, in catabolic conditions like physical exercise, GH predominantly stimulates the release and oxidation of FFA, which leads to decreased oxidation of glucose and proteins as well as preservation of lean body mass and glycogen stores [3]. This is consistent with the “feast and famine cycle” by Rabinowitz and Zierler [4] suggesting that insulin is the most important anabolic hormone directing all fuels to be stored during feast. In contrast, GH is implicated as an anabolic hormone during stress, sparing glucose and protein at the expense of lipids [4]. Indeed, using stable isotope methodology, Gibney et al. have shown that in patients with GH-deficiency (GHD) systemic lipolysis is decreased and uptake of FFA into tissue is reduced during a short-term exercise bout until exhaustion [5].

The supply of FFA to working tissue such as skeletal muscle is mainly fueled by lipolysis of lipids in adipose tissue [6] or from locally stored lipid depots, such as the intramyocellular lipids (IMCL) [7,8]. IMCL belong to the so-called ectopic lipids, which include - in addition to the IMCL – the intrahepatocellular lipids (IHCL). While several studies have shown that IMCL are depleted during exercise in healthy subjects [9–21] and in patients with type 1 diabetes mellitus [22] or GHD [23,24], previous data also suggest that IHCL increase immediately after a bout of exercise indicating that both, IMCL and IHCL, are flexible fuel stores [25].

The localization of IMCL as lipid droplets next to mitochondria

* Corresponding author at: Division of Endocrinology, Diabetology and Metabolism, University Hospital of Basel, Petersgraben 4, 4031 Basel, Switzerland.
E-mail address: emanuel.christ@usb.ch (E. Christ).
[1] These authors have equally contributed to this manuscript.

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suggest that IMCL are muscular fuel stores that can be used as energy source during physical exercise thereby reducing the need for uptake of systemically released FFA [8]. Indeed, several studies have shown that endurance-trained athletes exhibit increased IMCL levels that can be depleted by physical exercise and repleted by dietary intervention [6,13,26,27]. In contrast, overweight patients also present with high IMCL levels [28], but show a decreased capacity to deplete their IMCL [25]. Interestingly, patients with GHD deplete their IMCL to a similar degree compared with gender, age and BMI matched control subjects [23,24]. While in healthy subjects repletion of IMCL occurs within 24 h after the bout of exercise and depends on the intake of dietary fat [6,13,29], it is currently unknown whether the decreased lipolysis in patients with GHD during exercise influences ectopic lipid dynamics after exercise.

In contrast to IMCL, IHCL increase, rather than decrease, after an acute bout of exercise [25]. Most likely these findings are related to a transitory hepatic storage as a spillover of the increased availability of FFA during exercise. It is speculated that IHCL decrease 24 h after exercise since lifestyle interventions including exercise have shown to decrease IHCL [25] in parallel with a consistent decrease in fasting triglycerides (TG) and very-low-density-triglyceride (VLDL-TG) concentrations [30–33]. The decrease in IHCL has been shown to occur after 3–6 months [25] whereas the decrease in TG and VLDL-TG already occurs 20 h after an acute exercise bout [34]. Whether IHCL decrease already after 24 h and whether GHD influences post-exercise kinetics of IHCL is not established.

Using MR-spectroscopy, we, therefore, aimed at investigating ectopic lipids (IMCL, IHCL) in patients with GHD and controls subjects (CS) before and immediately after an acute bout of exercise as well as 24 h after exercise.

2. Material and methods

This is a prospective single-center trial performed at the University Hospital of Bern, Switzerland. The trial was approved by the local review board (Kantonale Ethikkommission Bern) and all subjects gave written informed consent. The study was performed according to the declaration of Helsinki, the guidelines of good clinical practice and Swiss health laws on clinical research. This trial was performed under the following clinical trial number NCT01467193.

2.1. Study participants

Seven male patients with severe GHD and seven male sedentary control subjects (CS) matched for age, BMI and waist circumference were included. Severe GHD was defined according to the current guidelines either based on an increase of GH to < 5.1 ng/ml during an insulin tolerance test (ITT) with a nadir plasma glucose of < 2.2 mmol/l and hypoglycemic symptoms, a pathological GH releasing hormone (GHRH)/arginine test with body mass index (BMI)-dependent cut offs (11.5, 8.0 and 4.2 ng/ml for BMI < 25, 25–30 and > 30 kg/m^2 respectively), or insufficiency of at least three pituitary axes in addition to a low value for insulin-like growth factor 1 (IGF-1) [35–37]. None of the patients has been treated with GH replacement therapy before.

Patients were included if they were capable of performing exercise on a bicycle ergometer for 2 h and had been under stable conventional hormone replacement therapy (glucocorticoids, thyroxin and testosterone) as needed for at least 6 months. Exclusion criteria were (former or present) ACTH- or GH-secreting pituitary adenoma, severe cardiovascular disease, diabetes mellitus, abnormal liver or renal function, active neoplasia, hemophilia, and therapy with drugs known to affect lipid or glucose metabolism or inability to exercise.

2.2. Study protocol

All the volunteers attended the research unit for three visits on three different dates. The minimal time between the first and the second visit was 7 days, the maximum 10 days. The third visit was always scheduled on the day following the second visit.

2.2.1. Determination of baseline characteristics and VO_{2max} (Visit 1)

Participants attended the endocrine investigation unit after an overnight fast and having restrained from physical activity for 72 h before the test. End-expiratory waist circumference was measured with a flexible tape placed on a horizontal plane at the level of the iliac crest. A blood sample was taken by venipuncture (insulin, glucose, FFA total cholesterol, LDL-Cholesterol, HDL-Cholesterol, triglycerides, TSH, fT4, fT3, total testosterone, FSH, LH, Cortisol, IGF-1). Maximal aerobic exercise capacity was determined during an incremental workload test on a bicycle ergometer as previously reported [9].

2.2.2. Two-hour physical exercise on a bicycle at 50% of VO_{2max} (Visit 2)

Three days preceding the second visit all subjects refrained from physical activity and adhered to a standardized fat rich diet including additional fat intake of 0.75 g fat/kg body weight, in order to replete IMCL [38]. GHD patients and CS attended the hospital after an overnight fast. In the morning before the test, they received a standardized light meal consisting of 2 deciliter of orange juice, yoghurt, curd and cereals. Ectopic lipids (IMCL and IHCL) were measured using ^{1}H-MR-spectroscopy and visceral (VAT) and subcutaneous (SCAT) adipose tissue were separately assessed. Hydrocortisone replacement therapy was administered as needed 30 min before physical exercise. Subsequently, patients and CS exercised on a bicycle for 2 h at 50% of VO_{2max}. During the exercise, blood samples were taken from an indwelling catheter every 30 min to determine serum glucose, FFA, insulin, cortisol, GH, norepinephrine and epinephrine concentrations. Following the exercise, IMCL and IHCL were re-assessed. VLDL-TG concentrations were measured before and immediately after physical exercise and 24 h after physical exercise.

In the evening after visit 2 the subjects had a standardized meal consisting of 2 deciliter of orange juice, yoghurt, curd and cereals. The following morning, subjects received a standardized breakfast (identical content as before). After attending the research unit, another blood sample was taken and the third MR-Spectroscopy was performed (re-assessment of IMCL and IHCL).

2.3. Biochemical analysis

Plasma glucose concentration levels were measured by an enzymatic hexokinase method (Roche, Modular P800). Insulin concentrations were measured with Architect, Abbott, Baar, Switzerland. Insulin sensitivity was estimated using homeostasis model assessment (HOMA, glucose × insulin/22.5) [40]. FFA concentrations were determined using a commercially available kit (Wako Chemicals, Dietikon Switzerland). TG, total cholesterol (TC) and LDL-C, (Hitachi 917, Roche, Rotkreuz, Switzerland). Cortisol concentrations were measured with Modular (Roche, Rotkreuz, Switzerland). Serum IGF-1 and GH concentrations were determined using a chemiluminescent immunometric assay (Immulite, Siemens, Zürich, Switzerland). Total testosterone concentrations were analyzed using Beckman Coulter RIA (IM1119). Plasma catecholamines (norepinephrine and epinephrine) concentrations were analyzed using ultraperformance liquid chromatography-tandem mass spectrometry (Waters Acquity UPLC/TQD, Manchester, UK).

Isolation of lipoprotein subfractions, including VLDL and measurements of VLDL-TG were performed as previously described [41].

2.4. MR measurements

All magnetic resonance examinations were performed on a Siemens Verio 3 T MR system as previously described [9].