METABOLISM CLINICAL AND EXPERIMENTAL 63 (2014) 1031–1040

Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Lipid droplet-associated proteins in high-fat fed mice with the effects of voluntary running and diet change

Rita Rinnankoski-Tuikka[a], Juha J. Hulmi[a], Sira Torvinen[a], Mika Silvennoinen[a], Maarit Lehti[b], Riikka Kivelä[a], Hilkka Reunanen[c], Urho M. Kujala[d], Heikki Kainulainen[a,*]

[a] Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland
[b] LIKES Research Center for Sport and Health Sciences, Jyväskylä, Finland
[c] Department of Biological and Environmental Sciences, University of Jyväskylä, Jyväskylä, Finland
[d] Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland

ARTICLE INFO

Article history:
Received 3 March 2014
Accepted 22 May 2014

Keywords:
Skeletal muscle
Insulin resistance
Perilipins
Physical activity

ABSTRACT

Objective. The relation between lipid accumulation and influence of exercise on insulin sensitivity is not straightforward. A proper balance between lipid droplet synthesis, lipolysis, and oxidative metabolism would ensure low local intramyocellular fatty acid levels, thereby possibly protecting against lipotoxicity-associated insulin resistance. This study investigated whether the accumulation of triglycerides and lipid droplets in response to high availability of fatty acids after high-fat feeding would parallel the abundance of intramyocellular perilipin proteins, especially PLIN5. The effects on these variables after diet change or voluntary running exercise intervention in skeletal muscle were also investigated.

Methods. During a 19-week experiment, C57BL/6J mice were studied in six different groups: low-fat diet sedentary, low-fat diet active, high-fat diet sedentary, high-fat diet active and two groups which were high-fat sedentary for nine weeks, after which divided into low-fat sedentary or low-fat active groups. Myocellular triglyceride concentration and perilipin protein expression levels were assessed.

Results. We show that, concurrently with impaired insulin sensitivity, the expression level of PLIN5 and muscular triglyceride concentration increased dramatically after high-fat diet. These adaptations were reversible after the diet change intervention with no additional effect of exercise.

Conclusions. After high-fat diet, lipid droplets become larger providing more surface area for PLIN5. We suggest that PLIN5 is an important regulator of lipid droplet turnover in altered conditions of fatty acid supply and consumption. Imbalances in lipid droplet metabolism and turnover might lead to lipotoxicity-related insulin resistance.

Abbreviations: BSA, Bovine serum albumin; DC, Diet change; DCA, Diet change active; DCS, Diet change sedentary; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; HF, High-fat; HFA, High-fat active; HFS, High-fat sedentary; HOMA-IR, Homeostasis model assessment of insulin resistance; IMTG, Intramyocellular triglyceride; LD, Lipid droplet; LF, Low-fat; LFA, Low-fat active; LFS, Low-fat sedentary; NEFA, Non-esterified fatty acid; PLIN, Perilipin; TG, Triglyceride.

* *Corresponding author at:* Department of Biology of Physical Activity, University of Jyväskylä, P.O. Box 35, FIN- 40014 Jyväskylä, Finland. Tel.: +358 50 330 2901; fax: +358 14 260 2001.
E-mail address: heikki.kainulainen@sport.jyu.fi (H. Kainulainen).

http://dx.doi.org/10.1016/j.metabol.2014.05.010
0026-0495/

1. Background

In conditions of high energy supply, ectopic fat storage exceeds intramyocellular demand and may eventually result in the development of skeletal muscle insulin resistance (for review, see [1]). Several recent studies have made mechanistic advances into the cause of insulin resistance as this relates to altered neutral lipid storage (for review, see [2]). As intramyocellular triglycerides (IMTG) are stored in lipid droplets, it has been hypothesized that high rates of IMTG oxidation allow regular turnover of the intramuscular lipid pool [3]. In contrast, non-utilization of the fatty acid reservoir in parallel with an increased amount of IMTG is linked with the deleterious effects of accumulating metabolically active lipid intermediates, such as long-chain acyl-CoA, diacylglycerol, ceramides and acylcarnitines, which are believed to blunt insulin sensitivity in skeletal muscle [2–4]. Currently, research interest seems to be shifting toward lipid droplet (LD) dynamics and imbalances in lipid metabolism. Thus, the role of LD coat proteins, which are essential in intramyocellular lipid storage and turnover as well as protection against lipid-induced insulin resistance, is being intensively explored [5].

The perilipin (PLIN) protein family is the best-characterized group of LD coat proteins [5]. This five-membered PLIN protein family shows considerable variation in tissue distribution between members, but all are involved in lipid accumulation and metabolism in LDs [6,7]. Perilipin 5 (PLIN5, also known as lsdp-5, MLDP, PAT-1 and OXPAT) is the most recently discovered member of the perilipin family. PLIN5 content seems to be proportional to LD content, with most abundant expression in tissues with high oxidative capacity, e.g. skeletal muscle [8–10]. The other two PLIN proteins investigated in this study are PLIN2 (also known as ADRP, ADFP and adipophilin) and PLIN3 (also known as Tip47, M6PRBP1 and PP17). Of these three PLIN proteins, ubiquitously expressed PLIN2 is found to be constitutively bound to LDs, whereas PLIN3 and PLIN5 are so called exchangeable proteins and can be found on both the lipid droplet and the cytosol [6,11]. The research field of LD-coating proteins is as yet rather sparse, but there is accruing evidence that these proteins are involved in LD dynamics, including LD synthesis, growth and fusion, triglyceride metabolism, organelle interactions, and intracellular trafficking [5,12]. Whether PLIN proteins correlate with energy balance or insulin sensitivity is still under debate. Modifications in dietary fat and exercise training can promote changes in IMTG accumulation [13,14], but the effects of such interventions on expression of PLIN proteins have only been sporadically investigated.

The aim of this study was to determine whether the accumulation of LDs in response to high availability of fatty acids after high-fat feeding would parallel the abundance of perilipin proteins PLIN2, PLIN3 and especially PLIN5 within skeletal muscle. Additionally, parallel effects on some of the key actors in IMTG lipolysis and lipogenesis, as well as in fatty acid transport were investigated. We also aimed to investigate how the aforementioned variables are affected by diet change or exercise intervention.

2. Methods

2.1. Animals and diets

Body weight-matched (n = 88) male C57BL/6J mice (Taconic Europe, Ejby, Denmark) were allocated to one of six intervention groups: The groups of low-fat fed (LF) and of high-fat fed (HF) mice were either sedentary (LFS, n = 14 or HFS, n = 14) or physically active (LFA, n = 15 or HFA, n = 15) throughout the experiment (Fig. 1). In addition, two further groups were first fed the HF diet and were sedentary for 9 weeks, after which their diet was changed to the LF diet (DC). One of these two groups remained sedentary (DCS, n = 15), while the other group had access to running wheels (DCA, n = 15). The mice were housed individually in standard conditions and the physically active mice had access to a running wheel, as previously described [15]. The mice received *ad libitum* for 19 weeks either a lard-based purified high-fat diet (61% E fat; D12492-Euro, Purina Mills TestDiet®, PMI® Nutrition International, Richmond,

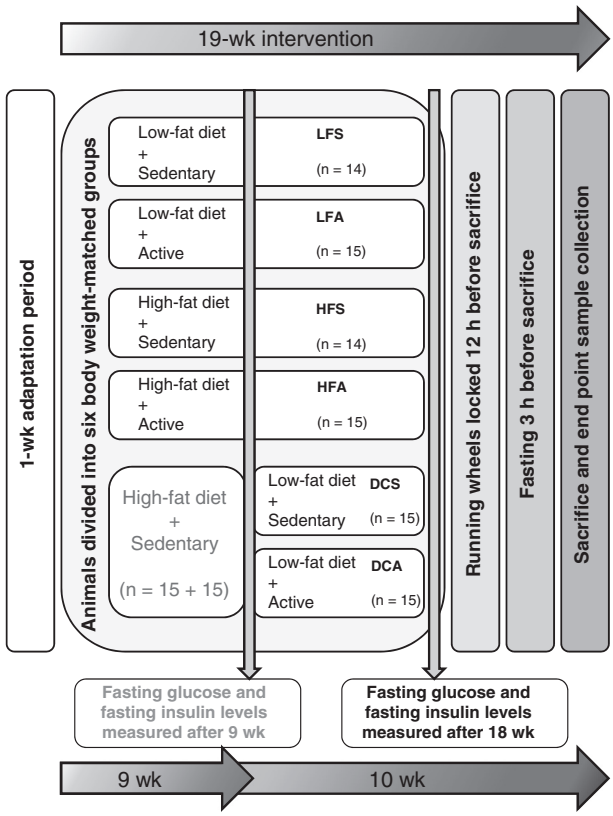

Fig. 1 – Summary of study set up. Graph summarizing the experiment set up and data collection points. During a 19-week experiment, C57BL/6J mice were studied in six groups: low-fat diet sedentary, low-fat diet active, high-fat diet sedentary, high-fat diet active and two groups, which were high-fat sedentary for nine weeks, after which divided into low-fat sedentary or low-fat active groups. Group size varied from 14 to 15 animals. LFS = low-fat sedentary, LFA = low-fat active, HFS = high-fat sedentary, HFA = high-fat active, DCS = diet change sedentary, DCA = diet change active.