Short-term HFD does not alter lipolytic function of adipocytes

Michael SF Wiedermann1,2,3, Stephan Wueest1,2, Alexandra Grob1,2,4, Flurin Item1,2, Eugen J Schoenle1,2, and Daniel Konrad1,2,3,*

1Division of Pediatric Endocrinology and Diabetology; University Children’s Hospital; Zurich, Switzerland; 2Children’s Research Center; University Children’s Hospital; Zurich, Switzerland; 3Zurich Center for Integrative Human Physiology; University of Zurich; Zurich, Switzerland; 4Institute of Human Movement Sciences; ETH Zurich; Zurich, Switzerland

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Three to four days of HFD suffice to induce hepatic insulin resistance, hepatic steatosis, and adipose tissue inflammation in susceptible mouse strains.1,4 However it remains debatable whether the acute dietary lipid overload, increased FFAs mobilized from endogenous fat depots, or inflammatory cytokines—all known to have causal roles in above mentioned processes per se—are responsible for the observed metabolic changes. We recently provided evidence that adipose tissue inflammation and an early dysfunctional crosstalk between adipose tissue and liver participates in the development of hepatic insulin resistance.5 To this end, adipocyte-specific Fas/CD95-deficient (FasΔadipo) or backcrossed control (Fas+/−) mice on a C57BL/6J background were fed either standard chow or HFD (12% or 58% of calories derived from fat) for 4 d. Besides its well-known role in the regulation of apoptosis, Fas/CD95 can also induce non-apoptotic pathways.6 In particular, Fas activation mediates the secretion of pro-inflammatory cytokines3,4 and its adipocyte-specific expression may contribute to obesity-induced adipose tissue inflammation and hepatic insulin resistance, as well as hepatic steatosis.9 Feeding mice a HFD for four days induced Fas protein level in isolated adipocytes, mesenteric adipose tissue inflammation (i.e., TNFα expression), hepatic steatosis, and hepatic insulin resistance as assessed by hyperinsulinemic–euglycemic clamp.5 Of note, adipocyte-specific Fas depletion relieved adipose tissue inflammation and hepatic insulin resistance without altering the degree of hepatic steatosis. Accordingly, it was recently demonstrated that TNFα release from WAT explants was increased after three days of HFD feeding and that TNFα neutralization prevented the development of hepatic insulin resistance.7 These data suggest that adipose tissue inflammation and resulting dysfunctional adipose tissue–liver cross-talk as an early event in the development of HFD-induced hepatic insulin resistance. In particular, reducing white adipose tissue (WAT) inflammation by adipocyte-specific depletion of Fas/CD95 protected mice from developing hepatic insulin resistance but not hepatic steatosis. Herein, we expanded our previous work and determined the impact of four days of HFD on lipolytic activity of isolated adipocytes. Compared with chow-fed mice, the degree of basal and isoproterenol-stimulated free fatty acid (FFA) and glycerol release was similar in HFD-fed animals. Moreover, insulin’s ability to suppress lipolysis remained intact, suggesting retained insulin sensitivity. Despite unaltered lipolysis, circulating FFA concentrations were greatly increased in non-fasted HFD-fed mice. In conclusion, a short-term HFD challenge does not affect lipolytic function of adipocytes. The observed increase of circulating FFA levels in randomly fed animals may rather be the result of increased dietary fat supply.
groups. Moreover, neither distribution of adipocyte according to their cell size (Fig. 1B) nor average adipocyte diameter (Fig. 1C) was different between chow- and HFD-fed mice. Importantly, release of lactate dehydrogenase (LDH) was similar suggesting comparable viability of isolated adipocytes from chow-fed and HFD-fed animals (Fig. 1D).

Lipolytic activity of epididymal adipocytes isolated from fed mice was assessed next. As depicted in Figure 2A and B, neither basal nor isoproterenol-stimulated lipolysis did differ between chow-fed and HFD-fed animals. In addition, the fold increase in isoproterenol-induced FFA release (chow 6.9 ± 1.8-fold vs. HFD 8.0 ± 2.9-fold; \( P = 0.75 \)) and glycerol release (chow 4.9 ± 0.8-fold vs. HFD 4.2 ± 0.5-fold; \( P = 0.43 \)) was similar, suggesting alike sensitivity to isoproterenol. Comparable basal and isoproterenol stimulated FFA and glycerol release suggested a similar degree of re-esterification. Indeed, calculations according to Rosenstock et al.\textsuperscript{12} revealed similar degrees of re-esterification for both basal (chow 33.7 ± 12.5% vs. HFD 51.5 ± 9.8%; \( P = 0.30 \)) and isoproterenol-stimulated (chow 17.3 ± 4.9% vs. HFD 24.3 ± 6.2%; \( P = 0.41 \)) lipolysis in both groups. Moreover, the ability of insulin to inhibit lipolysis (Fig. 2C) and to stimulate Akt phosphorylation (Fig. 2D) remained intact in adipocytes of HFD-fed mice suggesting no deterioration of insulin sensitivity in the latter. Even though lipolytic activity was similar between chow- and HFD-fed animals, circulating FFA levels were significantly increased in the latter in the fed state (Fig. 2E) whereas glycerol and triglyceride levels were not different (Fig. 2F and G). Similarly, increased FFA levels were previously reported for short-term HFD-fed non-fasted rats.\textsuperscript{13,14} However, after five hours of fasting FFA concentrations were similar between chow- and HFD-fed mice (Fig. 2H). Likewise, FFA levels were found to be no longer different between chow- and short-term HFD-fed animals after various periods of fasting,\textsuperscript{13,15-17} i.e., circulating FFA levels did increase in chow-fed animals during fasting to match FFA concentrations observed in HFD-fed animals. Of note, similar levels of phospho-HSL and phospho-perilipin were found in epididymal WAT after 5 h of fasting (Fig. 2I), suggesting similar lipolytic activity of WAT in both groups.

The fact that lipolysis was not affected by a short bout of HFD would suggest that increased circulating FFA levels in the fed state of short-term HFD-fed mice are rather the result of increased dietary fat supply. Interestingly, a short period of HFD sufficed to induce hepatic steatosis\textsuperscript{5,13} whereas it did not result in any significant increase in skeletal muscle and adipose fat accumulation as reported herein for adipose tissue and described previously for both skeletal muscle and/or adipose tissue.\textsuperscript{13,18} It is conceivable that the development of hepatic fat accumulation results from increased FFA flux into hepatocytes due to a dietary-induced rise in circulating FFA levels in the postprandial state. In this regard, a similar fat composition between the liver and

![Figure 1](link)
diet, both having an abundance of 18:2 fatty acid, was previously demonstrated. The evolving hepatic insulin resistance may then serve as early adaptation mechanism to protect the liver from further fat accumulation. As previously demonstrated we did not observe increased mRNA expression of macrophage or inflammatory markers in livers of mice fed a fat-enriched diet for four days. Moreover, as mentioned above, a reduction of adipose tissue inflammation had no impact on short-term HFD-induced...

Figure 2A–F (For G–K, see page 118). Similar lipolytic activity but increased non-fasted circulating FFA levels in short-term HFD-fed mice. Basal, isoproterenol-stimulated (A and B), and insulin-inhibited (C) FFA or glycerol release from adipocytes isolated from epididymal WAT of chow-fed (black bars) and HFD-fed (open bars) mice are depicted. Results are the means ± SEM of 4 independent experiments in non-fasted mice. (D) Insulin (1 U/kg) was injected i.p. in randomly fed mice. Epididymal WAT was harvested 15 min later and phosphorylation of Akt at Ser473 was determined and normalized to actin (n = 4). Representative blots are shown. FFA (E), glycerol (F), as well as triglycerides (G) levels were determined in systemic blood plasma of non-fasted mice, n = 4. (H) FFA levels were determined in systemic blood plasma in mice fasted for 5 h, n = 6. (I) Phospho-HSL (Ser660) and phospho-Perilipin (Ser522) protein levels were determined in epididymal WAT harvested from chow-fed (black bars) and HFD-fed (open bars) mice fasted for 5 h (n = 6) and normalized to actin. Representative blots are shown. FATP1 (J) and GLUT4 (K) content was analyzed in epididymal WAT harvested from randomly fed mice (n = 4). Representative blots are shown. All error bars represent SEM *P < 0.05 and ***P < 0.001 (Student t test), †P < 0.05 (isoproterenol vs. basal; Mann–Whitney U test).
hepatic steatosis but improved hepatic insulin sensitivity. Thus, such results would suggest that short-term HFD-induced hepatic insulin resistance is not the result of hepatic inflammation or hepatic fat accumulation. Of note, insulin sensitivity of skeletal muscle and adipose tissue was retained after 4 d of HFD as shown herein and as was previously reported.1,3,5,13 The anticipated increase in circulating insulin levels due to hepatic insulin resistance19 may enhance insulin-stimulated glucose uptake into adipose tissue and, thus, evolving fat accumulation.

Potentially, reduced fatty acid uptake by adipocytes may lead to increased circulating FFA levels (due to reduced FFA flux into adipocytes) and hence, to reduced re-esterification. However, as depicted in Figure 2A, FFA levels in the incubation media of adipocytes of chow- and HFD-fed mice were similar, as was the

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**Figure 2G–K (For A–F, see page 117).** For figure legend, see page 117.
degree of re-esterification. In addition, protein abundance of the long-chain fatty acid transporters FATP1 (ACSVL5) was even increased in adipose tissue of HFD-fed mice (Fig. 2J). Hence, such data would suggest that there is no impaired capacity of FFA uptake into adipocytes of HFD-fed mice. Likewise, basal and insulin-stimulated glucose uptake does not appear to be affected by short-term HFD as indicated by similar GLUT1 (not shown) and increased GLUT4 (Fig. 2K) content as well as preserved insulin-stimulated Akt phosphorylation (Fig. 2D).

In conclusion, short-term HFD does not affect lipolytic activity of isolated adipocytes. The observed increase of circulating FFA levels in randomly fed animals after short-term high fat feeding may rather be the result of increased dietary fat supply.

Materials and Methods

Animals

C57BL/6J mice were originally obtained from The Jackson Laboratory and then bred in our own facility. At the age of 12 wk animals were fed ad libitum with standard rodent diet (chow) or HFD (D12331, Research Diets) for 4 d. HFD consisted of 58% of calories derived from fat, 25.5% from carbohydrates, and 16.5% from protein. For additional experiments, mice were not fasted prior to sacrifice to be able to analyze adipocytes in a fed (baseline) state as there seems to be a rapid change, e.g., in circulating FFAs with prolonged periods of fasting. 13 All protocols conformed to the Swiss animal protection laws and were approved by the Cantonal Veterinary Office in Zurich, Switzerland.

Determination of plasma glucose and insulin levels

Blood was sampled in mice fasted for 5 h. Blood glucose concentration was measured with a Glucometer (Accu-Check Aviva, Roche Diagnostics) with blood from tail-tip bleedings. Plasma insulin levels were measured using an ELISA kit as described previously. 20

Viability assessment and cell size determination

Adipocytes were isolated and viability was determined with an LDH assay as described previously. 21 Aliquots of isolated adipocytes were used to determine mean cell diameters. Cell size was analyzed by a Multisizer™ 3 Coulter Counter® as follows. Two hundred microliters of isolated adipocytes were incubated in the absence or presence of 100 nM insulin or 1 μM isotretofen (Sigma) for one hour. 21 FFA levels were measured using the ACS-ACOD-MEHA method from Wako Chemicals GmbH. Triglyceride and glycerol levels were determined using a colorimetric assay, as described. 9

Western blotting

Samples were homogenized as described previously.21 Protein concentration was determined using BCA assay (Pierce), and equivalent amounts of protein (30–40 μg) were resolved by LDS-PAGE (4–12% gel, NuPAGE; Invitrogen). Proteins were electro-transferred onto nitrocellulose membranes (0.2 μm; Bio-Rad) or PVDF membranes (0.45 μm; Roche Diagnostics GmbH), and equal loading was confirmed by Ponceau S staining. The following primary antibodies were used: anti-actin was purchased from Millipore, anti-phospho-HSL (Ser660) as well as anti-phospho-Akt (Ser473) and total Akt from Cell Signaling Technology, anti-FATP1 from Santa Cruz Biotechnology, and anti-phospho-Perilipin (Ser522) from Vala Sciences. Primary antibodies against GLUT1 and GLUT4 were a kind gift from Dr Amira Klip, The Hospital for Sick Children, Toronto, Canada. Membranes were exposed in an Image Reader and analyzed with Image Analyzer (FujiFilm). Arbitrary values obtained with Image Analyzer were normalized to an average of 1 in the control chow-fed group.

Data analysis

Statistical analyses were performed using the Student t test or Mann–Whitney U test where appropriate. P values < 0.05 were considered significant. All error bars represent SEM.

Disclosure of Potential Conflicts of Interest

No conflict of interest existed for any of the authors.

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