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Activating cardiac E2F1 induces up-regulation of pyruvate dehydrogenase kinase 4 in mice on a short term of high fat feeding

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ABSTRACT

A high fat diet (HFD) induces substantial cardiac metabolic alteration(s), but the initiating molecular events remain unclear. We assessed the early cardiac energy metabolic changes in C57/BJ mice fed a HFD for 10 days. Carbohydrate oxidation was markedly decreased in mice on a HFD, in which up-regulation of pyruvate dehydrogenase kinase 4 (PDK4) was evident. Concurrently, E2F1, a transcription factor controlling PDK4 expression, was activated, as was cyclin D1, an upstream-molecule of E2F1, and eukaryotic initiation factor 4E (eIF4E), a modulator of cyclinD1 translation. Hence, HFD may initiate early cardiac metabolic alterations through the eIF4E/cyclin D1/E2F1/PDK4 axis.

1. Introduction

A high fat diet (HFD) can lead to insulin resistance and the development of cardiovascular diseases [1]. One of the HFD-induced metabolic alterations in the heart is an increase in fatty acid oxidation and a reduction in glucose utilization [2,3]. Competition between fatty acids and carbohydrates can occur at the level of the initial uptake of substrates into the cells, or at the subsequent transport into the mitochondria. At the level of the mitochondria, the pyruvate dehydrogenase complex (PDC) catalyzes the conversion of pyruvate to acetyl-CoA, thereby controlling glucose oxidation. Activity of PDC can be negatively modulated by pyruvate dehydrogenase kinase 4 (PDK4), that in turn, is regulated at the transcriptional level by multiple factors, including transcription factors, such as E2F1 [4], PPARα [5], PGC-1α [6], Foxo1 [7] and estrogen-related receptors [6].

The transcription factor E2F1, besides being a critical cell cycle regulator, also plays an important role in controlling mitochondrial function [8,9] and glucose homeostasis [4,10,11]. The activity of E2F1 is largely controlled by the phosphorylation of retinoblastoma (Rb), leading to dissociation of the Rb/E2F1 complex thereby releasing E2F1 for nuclear translocation [12,13]. Phosphorylating Rb (p-Rb), at a specific residue serine 780 (s780) by cyclin-dependent kinase 4 (CDK4) [14], requires elevated cytosolic cyclin D1 to form a complex with CDK4 [15]. Up-regulation of cytosolic free cyclin D1 can be regulated at the transcriptional level [16], but frequently occurs as a result of post-translational modifications [17,18]. A specific regulation of cyclin D1 translation by eukaryotic initiation factor 4E (eIF4E) has also been demonstrated [19,20].

Two overlapping binding sites of E2F1 on the PDK4 promoter have been identified [4]. Over-expression of E2F1 in C_2C_{12} myoblasts up-regulates PDK4 expression and suppresses glucose oxidation [4]. Conversely, inhibition of E2F1 prevents the development of myocyte hypertrophy in vitro [21], while loss of E2F1 in vivo blunts PDK4 expression and increases cardiac glucose oxidation [4].

The aim of this study is to explore how the HFD initiates the signalling pathways in mediating the myocardial energy metabolism in mice early in the course of obesity development.

2. Materials and methods

All animals received care and treatment according to Canadian Council on Animal Care and University of Alberta Health Sciences Animal Welfare Committee. Male C57BL/6 mice of 8 week of age were placed on either a HFD (60% calories from lard) or a low fat

Abbreviations: HFD, high fat diet; LFD, low fat diet; PDC, pyruvate dehydrogenase complex; PDK4, pyruvate dehydrogenase kinase 4; Rb, retinoblastoma; PPARα, peroxisome proliferator-activated receptor; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-α; CDK4, cyclin-dependent kinase 4; eIF4E, eukaryotic initiation factor 4E; eIF2α, eukaryotic initiation factor 2α; 4EBP1, eukaryotic initiation factor 4E-binding protein-1

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diet (LFD) (4% calories from lard, Research Diets; D12492) for 10 day or 3 week period. Hearts were excised and immediately frozen in liquid N_2 for biochemical analyses or perfused in the isolated working mode.

2.1. Heart perfusions

Isolated working hearts were perfused with Krebs–Henseleit solution containing 1 mM lactate, 5 mM glucose, 0.8 mM palmitate, 3% fatty acid free bovine serum albumin, and 2.5 mM free Ca^{2+}. To measure fatty acid oxidation and lactate oxidation rates, hearts were subjected to a 60 min perfusion with perfusate containing [9,10-^{3}H]palmitate and [U-^{14}C]lactate. To measure rates of glycolysis and glucose oxidation, another series of hearts were perfusated in Krebs–Henseleit solution containing [5-^{3}H] glucose and [U-^{14}C] glucose. Oxidation and glycolytic rates were determined simultaneously by quantitative collection of $^{14}CO_2$ and ^3H_2O produced by the hearts. The perfusions were performed at a left atrial preload of 11.5 mmHg and an aortic afterload of 50 mmHg. Heart rate, cardiac output, and cardiac work were measured as described previously [22].

2.2. Assessment of β-hydroxylacyl CoA dehydrogenase and citrate synthase activity

The activity of β-hydroxylacyl CoA dehydrogenase and citrate synthase was measured based on the continuous spectrophotometric rate determination, following the reduction of NAD^+ at 340 nm [23] or the increased production of TNB at 412 nm [22], respectively.

2.3. Myocardial triacylglycerol (TG), malonyl-CoA, long chain-CoA, and ceramide determination

Myocardial TG was quantitated colorimetrically with an enzymatic assay (Wako Pure Chemical Industries) [22]. Myocardial malonyl-CoA, long chain-CoA and ceramide were assessed by HPLC [22].

2.4. Pyruvate dehydrogenase complex (PDC) activity

Measurement of PDC was performed using a radioisotopic-coupled enzyme assay following acetyl-CoA production, as previously described [24].

2.5. Antibodies for immunoblot analysis

Antibodies for phospho-Rb(s780), phosphor-cyclin D1(Thr 286)/total cyclin D1, eIF4E, phosphor-4EBP1, phosphor-eIF2α/total eIF2α and lamin A were purchased from Cell Signaling Technology (Beverly, MA). Antibodies for CDK4 and PDK4 were obtained from Abcam, while anti-E2F1 and anti-actin were from Santa Cruz Biotechnology. The immunoreactive bands were quantified by scanning densitometry with ImageJ image analysis software.

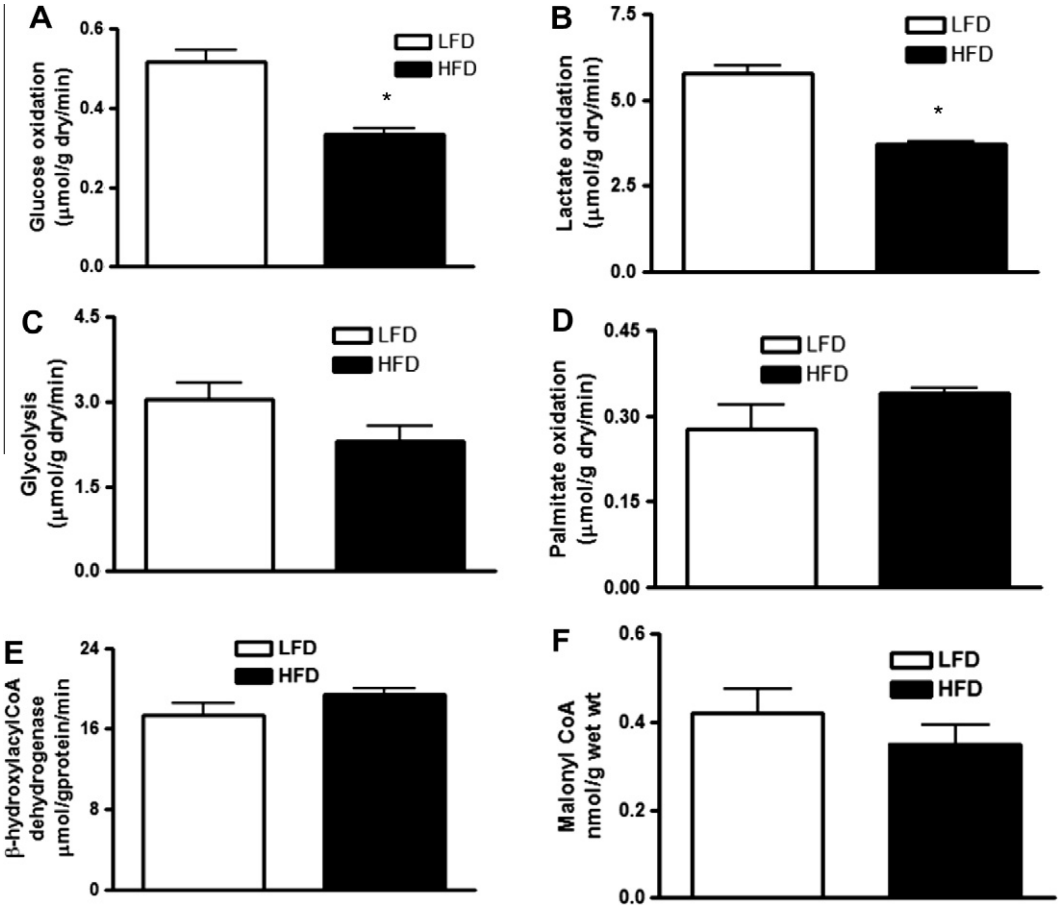

Fig. 1. Altered myocardial energy metabolism in mice fed a HFD for 10 days. In the isolated working hearts, the basal energy metabolism was assessed in the absence of insulin. (A) Rates of glucose oxidation. (B) Rates of lactate oxidation. (C) Rates of glycolysis. (D) Rates of palmitate oxidation. (E) Activity of β-hydroxylacyl CoA dehydrogenase. (F) Myocardial malonyl CoA level. Values represent mean ± S.E.M. ($n = 5$). *$P < 0.05$, significantly different from the LFD.