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# Effects of the activation of heme oxygenase-1 on hormonal and metabolic changes in rats fed a high-fat diet

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ABSTRACT

Heme oxygenase-1 (HO-1) is the inducible isoform of the heme oxygenase system, which catalyzes heme degradation. Up-regulation of this enzyme under pathological conditions is associated with beneficial effects in the whole organism. However, the potential of HO-1 in the alleviation of disturbances induced by a high-fat diet (HFD) is poorly elucidated. The present study was undertaken to determine the effects of pharmacological activation of HO-1 by hemin on some hormones and metabolic parameters in rats fed an HFD for 8 weeks.

It was demonstrated that, in rats fed an HFD, blood glucose levels were increased compared with control animals. However, this hyperglycemic effect was alleviated by induction of HO-1. The observed decrease in glycemia was not associated with an increase in blood insulin concentrations, but was accompanied by improved glucose tolerance, which points to the potentiation of insulin action. Concentrations of free fatty acids were elevated in response to HFD; however, this effect appeared to be mitigated by hemin. Rats fed an HFD displayed clear-cut hyperleptinemia, which is a hallmark of leptin resistance. This derangement was effectively prevented by hemin therapy. Feeding with an HFD also increased blood ghrelin levels, whereas hemin slightly reduced blood ghrelin concentration. Carbohydrate and lipid metabolism in the liver of rats on an HFD was found to be disturbed, leading to increased lipid accumulation and reduced glycogen stores. However, negative changes in liver metabolism were partially attenuated as a result of induction of HO-1.

Our results show that activation of HO-1 by hemin ameliorates some changes induced by HFD feeding. Normalization of blood leptin levels in these animals seems to be the most relevant finding, since hyperleptinemia is associated with dysregulation of energy homeostasis and with numerous other disorders. These results indicate that the HO system holds great potential to alleviate alterations induced by HFD.



## 1. Introduction

Heme oxygenase (HO) is a microsomal enzyme (EC 1.14.99.3), which catalyzes the oxidative degradation of heme. Three isoforms of HO have been identified – HO-1, HO-2 and HO-3. HO-1, also known as heat shock protein 32, is the inducible isoform and, therefore, is of great interest. HO-1 undergoes activation, among others, under conditions of hypoxia and oxidative stress. Moreover, increased activity of this enzyme is observed in animal tissues in response to some xenobiotics, radiation and inflammatory processes [1]. Animal studies show that up-regulation of HO-1 is beneficial, since enhanced degradation of heme generates more carbon monoxide and biliverdin, and these compounds are thought to protect cells against oxidative stress, inflammation and apoptosis [1]. Consistent with this notion, pharmacological induction of HO-1 has been demonstrated to have positive implications under pathological conditions. HO-1 exerts multifunctional roles in the cardiovascular system. Activation of this enzyme alleviates oxidative stress in ischemia and the resulting hypoxia-reperfusion injury. This protective action is particularly clear-cut in the heart [2]; however, up-regulation of HO-1 is also capable of protecting blood vessels from endothelial dysfunction [1,3]. Apart from cardio-vascular benefits, hepatoprotective [4,5] and renoprotective [6,7] actions of the HO system have been reported. This action is strongly linked with anti-inflammatory and anti-oxidative effects.

Studies with animal models have shown that induction of HO-1 is also beneficial in tackling obesity and diabetes. Given the

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increasing prevalence of obesity and diabetes worldwide, these properties seem to be particularly valuable. Excessive adipose tissue accumulation leads to people becoming overweight or obesity, and this is closely associated with dyslipidemia, the risk of insulin resistance and type 2 diabetes. The impairment in insulin action is partially due to exaggerated release of free fatty acids from adipose cells to the blood [8,9]. Moreover, it has recently been established that adipose tissue inflammation in obese subjects also plays a role in the pathogenesis of insulin resistance [10]. Obesity induces changes in the resident immune cells, which are present within adipose tissue and generate the inflammatory response [11]. Adipocytes also release multiple adipokines, which have important regulatory functions. However, the abnormal release and/or action of some adipokines, e.g. reduced blood adiponectin levels, impairs insulin action [10]. Activation of HO-1 has been demonstrated to have beneficial effects in Zucker diabetic fatty rats, in which obesity and insulin resistance are determined genetically [7,12,13], and in rats fed a high-fat diet and treated with streptozotocin [14], which selectively destroys pancreatic β-cells [15]. It has been demonstrated that, in mice fed a high fat diet, induction of HO-1 reduces adipose tissue inflammation via PPARγ and STAT6 pathways [16]. Recent research also shows that some diabetic complications may be reduced as a result of induction of HO-1 [17]. However, beneficial effects of HO-1 in diabetic animals are not only associated with fat tissue. Activation of this enzyme ameliorates diabetes in insulin-resistant GK rats, a non-obese model of type 2 diabetes [18,19], and in rats with streptozotocin-induced diabetes [20].

Induction of HO-1 in hyperglycemic rats reduces blood glucose levels [5,12,14,19–21]. Moreover, in rodents with insulin resistance, up-regulation of HO-1 improves insulin action [12,18,22]. Activation of this enzyme in diabetic rats is also associated with increased antioxidant capacity [14,19] and reduced inflammation [12]. In line with results from animal studies, a defective HO system has been reported in tissues of type 2 diabetic humans [23–25]. However, in spite of these data, very little is known about the potential benefits of the HO system in animals on a high-fat diet. The present study was undertaken to determine the effects of pharmacological activation of HO-1 on hormonal and metabolic changes in rats fed a high-fat diet.

## 2. Materials and methods

### 2.1. Chemicals

Hemin (chloro(protoporphyrinato)iron(III)), enzymes used to determine blood glucose and lactate levels and to determine liver glycogen and cholesterol content, dimethyl sulfoxide (DMSO) and other reagents were purchased from Sigma (St. Louis, MO, USA). Analytical kits to determine total protein content, albumin and β-hydroxybutyrate, to measure activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were obtained from Pointe Scientific.

### 2.2. Animals and experimental protocol

Male Wistar rats obtained from Brwinow (Poland) were used in the study. Animals were maintained in cages in an air-conditioned animal room at a constant temperature (21 ± 1 °C) and with a 12:12-h dark/light cycle. Rats with an initial body weight of 220 ± 20 g were divided into four groups (n = 8): control group, hemin-treated rats, rats fed an HFD, rats fed an HFD and treated with hemin. Subjects from the first and second groups were fed *ad libitum* a standard laboratory diet, whereas rats from the third and the fourth groups were kept on an HFD. The standard laboratory diet (Labofeed B, 3.5% of fat) and the high-fat diet (40% of fat) were from Kcynia (Poland). Hemin (30 mg/kg body weight), used to induce HO-1, was dissolved in a vehicle (DMSO: 0.1 M phosphate buffer, pH 7.4, 1:1) and was given intraperitoneally twice a week. The hemin solution or vehicle (2 ml/kg) were given for eight weeks. The dose of hemin used in the study had previously been shown to be sufficient to up-regulate HO-1 in rat tissues [5,12,18]. After eight weeks, animals were killed by decapitation (between 9 AM and 11 AM), and their blood serum, liver and soleus muscle were sampled and stored (−80 °C) until analysis. The last injections of hemin solution or vehicle were made 3 days before the end of the study. The experimental protocol was approved by the Local Ethical Commission for Investigation on Animals.

### 2.3. Blood hormone levels

Concentrations of insulin, glucagon, leptin and total ghrelin in blood serum were assessed by radioimmunoassay using kits provided by Millipore (St. Charles, USA).

### 2.4. Metabolic parameters, proteins and aminotransferase activities

Blood glucose levels were determined by the enzymatic method with glucose oxidase, peroxidase and dianisidine [26]. Concentrations of blood lactate were determined by the measurement of NADH formed from $NAD^+$ in the presence of lactate dehydrogenase [27]. Blood free fatty acids and triglycerides were analyzed colorimetrically by the method of Duncombe [28] and Foster and Dunn [29], respectively. Concentrations of total protein, albumin and β-hydroxybutyrate and activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in blood serum were determined using kits, according to instructions provided by the manufacturer.

In order to measure liver and muscle glycogen content, tissue samples were hydrolyzed in 30% KOH. Then, glycogen was decanted, decomposed in the presence of amyloglucosidase and determined as blood glucose. To determine the amounts of liver triglycerides and cholesterol, tissue samples were homogenized in chloroform: methanol solution (2:1) and total lipids were extracted according to the method described by Folch et al. [30]. Then, triglycerides were determined by the method of Foster and Dunn [29]. In the case of cholesterol, aliquots of chloroform: methanol extract were evaporated and total cholesterol was determined using cholesterol esterase and cholesterol oxidase [31].

### 2.5. Glucose tolerance test

One week before the end of the study, rats were fasted overnight and blood samples were taken from the tail vein to determine concentrations of glucose. Then, animals were given glucose solution intragastrically (2 g per kg body weight) and glycemia was determined 50 min later. Blood glucose levels were measured using a glucometer (HemoCue Glucose 201, Angelholm, Sweden).

### 2.6. Statistical analysis

The obtained results represent the means ± SEM from 8 animals. The results were evaluated statistically using analysis of variance and Duncan's multiple-range test. Differences were considered statistically significant at $P < 0.05$.

## 3. Results

### 3.1. Effects of hemin and HFD on blood hormone levels

Feeding rats an HFD and/or hemin treatment for eight weeks was associated with significant hormonal changes. Treatment of