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# Expression and Inflammatory Regulation of Haptoglobin Gene in Adipocytes

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**Summary:** Haptoglobin (HP) is the major hemoglobin binding protein which is synthesized mainly in the liver. It functions to prevent iron loss and kidney damage in human and other mammals. Recently, HP has been shown to possess antioxidant and angiogenic properties. As one of the major acute phase reactants, HP's levels in plasma increase significantly during inflammation, infection and malignancy. In this study, high levels of HP mRNA were found to be transcribed by adipocytes in addition to liver cells in mice. After inflammation had been induced *in vivo*, expression of the haptoglobin gene rose six-fold in adipose tissue, an increase compatible with that observed in the normal mouse liver. The expression of HP by adipocytes presents new directions in which HP's role as an antioxidant or as an angiogenic factor can be investigated.


In mammals, the systemic response to disturbed homeostasis, the acute phase reaction, is characterized by a dramatic alteration in the hepatic synthesis of a group of plasma proteins, known as acute phase proteins (1, 2, 3). A common feature of many acute phase proteins is their involvement in host defense against infection and in repair of damaged tissues, thereby restoring homeostasis. Although acute phase proteins are synthesized mainly in the liver, extra-hepatic expression of these proteins has been reported (4, 5, 6).

Haptoglobin (HP) is the major hemoglobin-binding protein in humans and other mammals. By forming a complex with hemoglobin, HP prevents both iron lost and kidney damage during hemolysis (7, 8). HP has also been shown to inhibit hemoglobin-stimulated lipid peroxidation and it has been suggested to have an important antioxidant function *in vivo* (9). Recently, Cid et al. (10) found that HP functions as an angiogenic factor. Purified HP stimulates endothelial cell differentiation and vascularization in a dose-dependent manner in *in vivo* and *in vitro* models. Since HP expression is up regulated during inflammation, it is possible that HP might have a role in repairing damaged capillaries.

Although HP is one of the few acute phase proteins whose increased synthesis during inflammation is conserved in all vertebrate species studied (1), the role(s) and the regulation of

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HP synthesis in extra-hepatic tissues are poorly understood. In this study, we have identified adipose tissue as a major site of extra-hepatic HP mRNA synthesis in mice. We have demonstrated that fat cells in adipose tissues are important cell types that synthesize haptoglobin, and that HP expression increases significantly when inflammation is induced. The possible contribution of HP synthesis in adipose tissues is discussed.

## Materials and Methods

***Source of tissues and isolation of cells:*** Tissues and cells were derived from C57BL6 mice. Inflammation was induced in mice by injecting 4.5 μg of lipopolysaccharide (LPS, Sigma ) per gram of body weight into the peritoneal cavity. Tissues and cells were collected 24 or 30 hours after LPS or saline injection. Adipocytes and non-fat cells were isolated from the epididymal fat pad of the mouse by sedimentation according to the method described by Bjorntorp et al. (11).
***Isolation of RNA and Northern blot analysis:*** Total cellular RNA was extracted from C57BL6 mouse tissues by homogenizing the tissues in guanidinium thiocyanate followed by phenol-chloroform extraction (12). Northern blot analysis was performed as described by Fourney et al. (13). Mouse HP cDNA (14) insert was $^{32}$P-labeled by the random priming procedure (15) and was used as a hybridization probe. Quantitative analysis of hybridization signals was conducted by using a Model 603 blot analyzer (Betagen Corp.). Relative amounts of actin mRNA and/or rRNA in each gel slot were used as gel loading controls.
***In Situ hybridization histologic study:*** Mouse tissues were quick-frozen in Tissue-Tek OCT compound in dry ice. Sections of 5μm thick were prepared. Fixation and hybridization of cryosections were performed as previously described by Zeller and Rogers (16). $^{35}$S-labeled sense and antisense riboprobes were synthesized using linearized mouse HP 1-1 cDNAs (14) in pGEM7zf(+) plasmid as templates. For autoradiography, slides were coated with film emulsion (Kodak NTB-2) and exposed at 4-8 °C for 3-10 days. Slides were then developed with Kodak D19 developer and stained with hematoxylin and eosin.

## Results

In Figure 1, 1 μg of poly(A)+ RNA prepared from adult (2-3 month-old) mouse liver and 10 μg of poly(A)+ RNA isolated from other adult mouse organs were compared by Northern blot analysis. In addition to strong expression of HP in liver, high amounts of HP mRNA were detected in adipose tissue and lung indicating that these two tissues are major extra-hepatic sources of haptoglobin. In normal healthy (control) animals, the levels of HP mRNA in adipose were calculated in several experiments, including those shown in Fig. 3, to be approximately 10-15% of the levels in liver. Low levels of HP mRNA were found in ovary, uterus, submaxillary and adrenal glands.

*In situ* hybridization analyses were performed on tissue sections from different organs to determine the cellular origin of HP mRNA. Among the mouse organs and tissues examined were: adrenal gland, skin, muscle, brain, epididymis, liver, lung, thymus, spleen, lymph nodes, esophagus, stomach, intestine, kidney, placenta, ovary, uterus and testes. In addition to hepatocytes, localization of HP mRNA was detected mainly in adipocytes and lung airway epithelium (Yang et al., unpublished data). HP mRNA was also observed in small numbers of

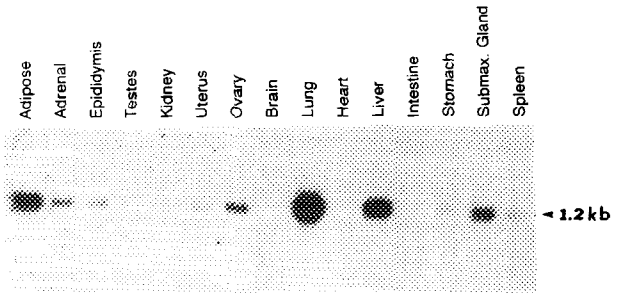


**Fig. 1. Detection of HP mRNA in tissues from adult mice.** Northern blot analysis was performed with 1 μg of poly(A)+ RNA from liver and 10 μg poly(A)+ RNA from other mouse tissues. Mouse HP cDNA insert, labeled with $^{32}P$, was used as a hybridization probe.

specific cells in adrenal, submaxillary gland, ovary and uterus as revealed by *in situ* analysis. The low levels of HP mRNA detected in other organs by Northern blot analysis were attributed mainly to the adipose tissue associated with these organs and not to cells in the organs themselves. The expression of HP in adipose tissue was further examined in this study.

Although the adipocyte is the predominant cell type in adipose tissue, a small number of other cells such as fibroblasts, endothelial cells and smooth muscle cells are present in adipose tissue (17). As shown by *in situ* hybridization (Fig. 2), adipocytes are the cells responsible for HP mRNA expression in adipose tissues. HP mRNA was not detected in fibroblastic, smooth muscle or endothelial cells in adipose tissues or other organs.

To investigate the regulation of HP gene expression in adipose tissue during inflammation, RNAs were prepared from adipose tissues derived from both lumbar and epididymal fat pads collected from mice injected with saline and LPS, respectively. Results from Northern blot analysis, shown in Fig. 3, demonstrated that a significant increase of HP mRNA in adipose tissue occurs with the onset of inflammation induced by LPS *in vivo*. Thirty hours after the mice were injected with LPS, HP mRNA levels increased six-fold in the adipose tissue. The increased levels of HP mRNA observed in adipose tissues were comparable to that detected in purified adipocytes (Fig. 3, Lane A). This was supported again by *in situ* hybridization. Other cells did not have any significant expression of HP mRNA during inflammation (Fig. 4).

## Discussion

In this study, we have demonstrated that adipose tissue is one of the major sites of HP expression in mice and within adipose tissue HP expression is confined to adipocytes. Although HP is produced mainly in the liver, our results suggest that adipose tissue may contribute significantly to the presence of HP in body fluids as well as in the serum. A phylogenic study of

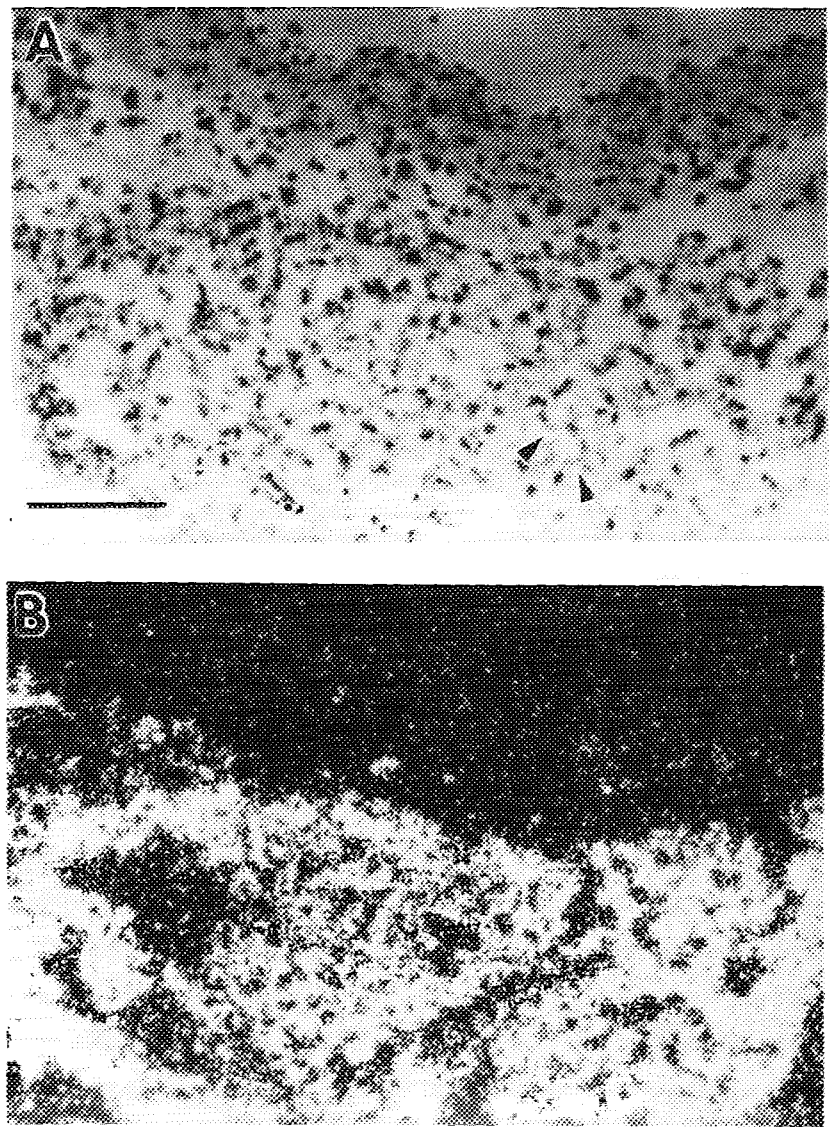


**Fig. 2.** ***In situ*** **hybridization of adipose tissue with antisense mouse HP cRNA probe.** This tissue section was derived from subserosal connective tissue with fat accumulations from a healthy adult male mouse. Silver grains were localized to the characteristic signature ring-shaped adipocytes (indicated by arrowheads). B is a dark field illumination of A. Scale bar = 0.1 mm.

HP expression in extrahepatic tissues will contribute to the understanding of the biological function of locally synthesized HP protein.

Haptoglobin increases approximately three- to ten-fold in the plasma of mice injected with LPS (18, 19). Regulation of haptoglobin's response to inflammation has been extensively studied *in vitro* in hepatoma cells (20, 21). HP gene is regulated by IL-6, IL-1, dexamethasone and

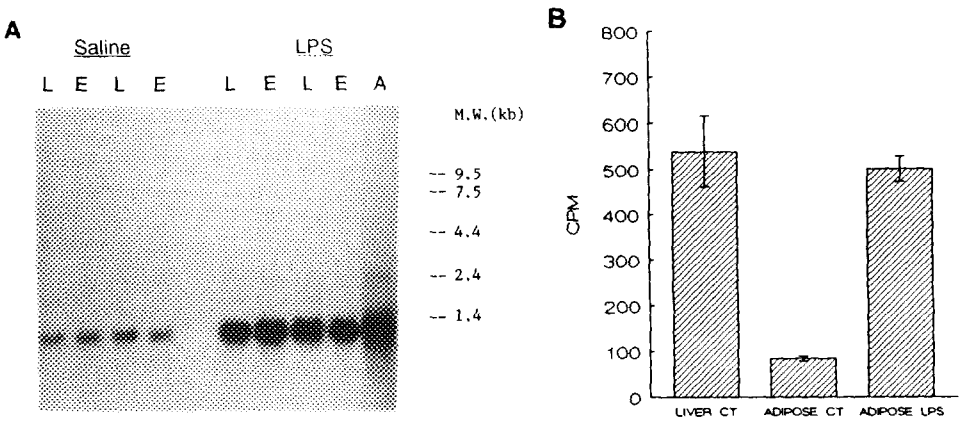


**Fig. 3. Northern blot analysis of HP mRNA in mouse adipose tissue and adipocytes.** In A, each lane contains 13 μg of total RNA prepared from either lumbar (lane L) adipose, epididymal (lane E) adipose or purified adipocytes (lane A) isolated from epididymal adipose tissue of adult male mice injected with either saline (left) or LPS (right) 30 hours before the animals were sacrificed. The result of the Northern blot analysis is also shown in a histogram (B) which includes the liver mRNA levels detected in 13 μg of total RNAs from 4 animals for comparison. CT = control (saline-injected).

TNFα in rat, but mainly by IL-6 and dexamethasone in human. The stimulatory effect on HP genes by IL-1 appeared to be different in different species of mice (19). IL-6, however, is the common inflammatory cytokine mediator for HP gene regulation in all species studied. The activation of HP genes as well as several acute phase genes has been attributed to the interaction between specific DNA sequences and the nuclear transcription factor, C/EBPβ (20, 22). C/EBPβ also is a major player in adipocyte differentiation (23). Expression of the HP gene in adipocytes has not been previously observed, even though fatty tissues have been used as a rich source for the extraction and typing of haptoglobin in the forensic science laboratories (24). The physiological role of haptoglobin in adipose tissue is of considerable interest, especially in inflammation. Adipocytes respond to specific hormonal and nutritional signals (25). Members of the C/EBP family, including the major inflammatory cytokine C/EBPβ, are expressed by adipocytes (23, 26). *In vitro* experiments have demonstrated that C/EBPβ and C/EBPδ are expressed early in adipocyte differentiation, but decrease after cell confluence (23). In adipocytes, gene expression varies significantly, depending upon the stage of cellular differentiation.

Genes expressed in adipocytes include both those related or unrelated to fat metabolism. In humans and rodents, adipose tissue transcribes high levels of the complement protease Factor D mRNA (27). Factor D, like haptoglobin, serves as a protein that has a protective role during inflammation, indicating that adipose may also be a source of fortifying factors unrelated to fat metabolism. The fatty acid binding protein and adipocyte P2 are other proteins synthesized by the

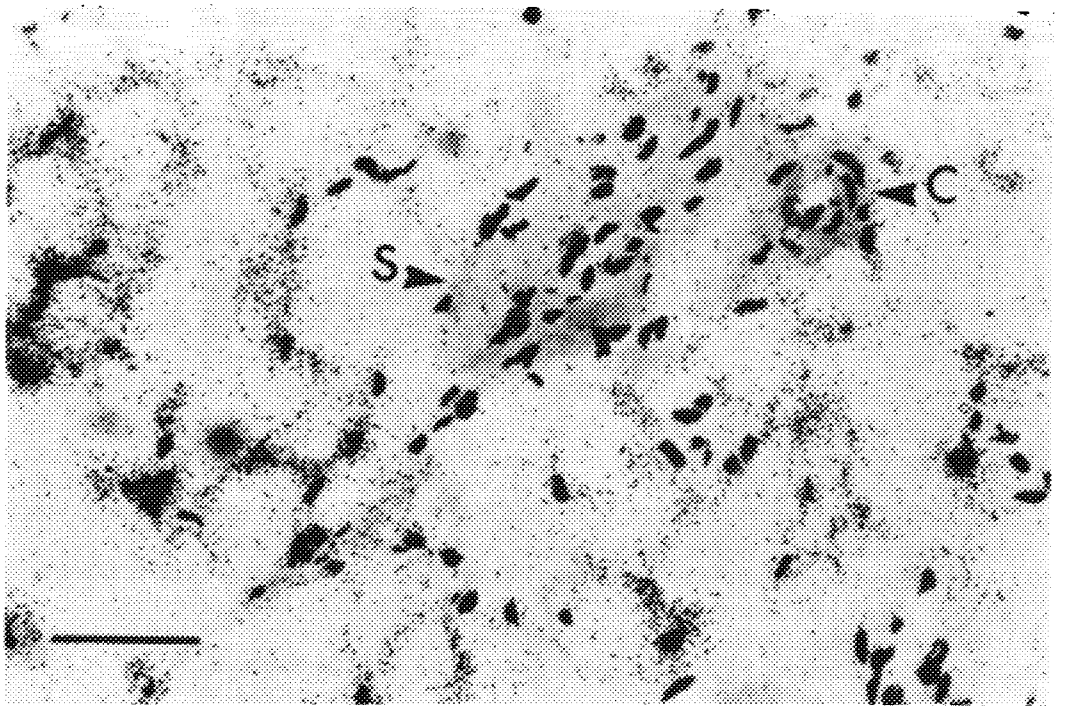


**Fig. 4. Localization of HP mRNA in adipocytes of adipose tissue after LPS treatment.** Hybridization signals were seen in ring-shaped adipocytes. No significant hybridization signal is seen in capillary (C) or smooth muscle cell (S). Scale bar = 0.05 mm.

differentiated adipocyte (25). The expression of haptoglobin by adipocytes and its modulation by inflammatory stimuli present new directions in which HP's role as an antioxidant or as an angiogenic factor can be investigated.

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