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Expression and Regulation of Haptoglobin in Tissues and in Differentiated Bovine Adipocytes

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Abstract

Haptoglobin (Hp) is a protein secreted mainly from the liver to bind hemoglobin and thus prevents iron loss and oxidative stress. Bovine Hp in blood increases during diseases such as mastitis and fatty liver syndrome. This study was conducted to examine the expression and regulation of Hp in differentiated mature bovine adipocytes. Hp expression was confirmed in various tissues and in mature adipocytes but not in the stromal vascular cell fraction using qualitative RT-PCR and quantitative northern blot analysis, respectively. Intravenous injection of Holstein cows with recombinant bovine tumor necrosis factor-α (rbTNF-α) and Interferon gamma (rbIFN-γ) significantly increased the serum concentrations of Hp. When mature differentiated bovine adipocytes were incubated with short chain fatty acids, cytokines and hormones, Hp expression was altered. Acetate, butyrate and propionate at a dose of 1 mM significantly up-regulated Hp mRNA

abundance. Lipopolysaccharide (LPS) and (TNF-α) also significantly up-regulated Hp mRNA abundance, while troglitazone and norepinephrine down regulated it. Our study confirmed expression of Hp in differentiated mature adipocytes and identified LPS, TNF-α, short chain fatty acids, as regulators of Hp mRNA expression in these cells.
Key words: Adipocytes; Bovine haptoglobin; Expression; Regulation

Introduction

White adipose tissue is an endocrine organ; it secretes various adipokines that play important roles in the regulation of both host defensive mechanisms and health status (Trayhurn and Beattie 2001). Several adipokines are involved in regulating glucose tolerance and insulin sensitivity (Mohamed-Ali et al., 1998). The importance of adipose tissue in the regulation of inflammation and defensive mechanisms during different diseases was shown in humans and various rodent species (Trayhurn and Beattie 2001). Haptoglobin (Hp) is an acute phase protein that is synthesized mainly by the liver with increasing secretion into the circulation during inflammation (Heinrich et al., 1990). Based on the expression of Hp in adipose tissue that was demonstrated in several monogastric species as recently reviewed by Ceciliani et al. (2012), Hp is also considered as an adipokine. In cattle, expression of Hp in extrahepatic tissues and organs such as mammary gland, leukocytes, forestomach and abomasum (Thielen et al., 2005 & 2007; Dilda et al., 2011) and also adipose tissue was demonstrated (Saremi et al., 2012). Haptoglobin binds hemoglobin and thus prevents iron loss and kidney damage (Putnam, 1975); it thereby reduces oxidative stress, preventing the generation of free radicals and lipid peroxides (Bertaggia et al., 2014). Beside its antioxidant properties, Hp has multiple functions, e.g. it promotes angiogenesis by stimulating endothelial cell differentiation and vascularization (de Kleijn et al., 2002; Quaye, 2008). Hp also exerts inhibitory effects on T-cell proliferation (Arredouani et al., 2003; Huntoon et al., 2008). Haptoglobin is also used as diagnostic marker for inflammatory states and infection: In cattle, the serum concentrations of Hp are below 20 μg/mL in healthy animals but increase by a factor of

100 to 1000 in response to immune stimulation (Godson et al. 1996). Hp is a good marker for various diseases such as mastitis and fatty liver syndrome in dairy cows (Eckersall and Bell 2010; Zarrin et al. 2014). In humans, secretion and expression of Hp have been demonstrated to be positively correlated with adiposity (do Nascimento et al. 2004; Doumatey et al. 2009), however, no such relationship could be established in dairy cows of normal body condition (Saremi et al., 2012). Expression of Hp in adipose tissue is increased in response to lipopolysaccharide (LPS) and to cytokines mediating the response to LPS in rodents (do Nascimento et al., 2004; Friedrichs et al., 1995).

The association of Hp with diseases such as diabetes, cardiovascular diseases and obesity has been manifold established in humans (Quaye, 2008). In 3T3L-1 adipocytes, Hp secretion and expression is under the control of various cytokines, catecholamines and peroxisome proliferator activator receptor-gamma (PPAR-γ) agonists (do Nascimento et al. 2004). Little about bovine haptoglobin expression and regulation in adipose tissue and differentiated adipocytes as reported by Saremi et al. (2010, 20012) and by our knowledge no data is available on its regulation *in vivo or in vitro***.** Therefore, this study was conducted to examine the tissue distribution of Hp expression and to characterize the effects of short chain fatty acids, cytokines, and other regulators on Hp mRNA expression in differentiated mature bovine adipocytes.

Materials and methods

Materials

Dulbecco's modified Eagles's medium (DMEM, DMEM/F12 and DMEM without glucose), Hank's balanced salt solution, bovine serum albumin (BSA), bovine insulin, isobutylmethylxanthine (IBMX), dexamethasone and sodium salt of short chain fatty acids (acetate, butyrate and propionate) were bought from Sigma-Aldrich Fine Chemical (St. Louis, MO, USA). Fetal calf serum (FCS) was from Trace Scientific Ltd. (Melbourne, Australia). Collagenase, lipopolysaccharide from *E. coli* (LPS), estradiol-17-β, norepinephrine and troglitazone (PPAR-γ agonist) were purchased from Wako Pure Chemicals Co. (Osaka, Japan).

Animals and Blood sampling

The experimental procedure and care of animals were in accordance with the guidelines of the Animal Care and Use Committee of Benha University, Egypt and Taif University, Saudi Arabia. Nine Holstein cows weighing 343.5 ± 28.5 kg were housed in individual stalls in the animal facility of Benha University with free access to water and trace mineral block, and given a mixture of forage (orchard-grass hay, alfalfa hay cube, corn silage) and concentrates (approximately 25 kg forage twice a day and 5 kg concentrates once a day). Three groups (3 cows per each group) non-pregnant, non-lactating Holstein cows were used for the experiment and were injected intravenously as follows: the first group was injected with phosphate buffer saline (PBS) as a control. The second group was injected with recombinant bovine tumor necrosis factor alpha (rbTNF-α) in a dose of 5μg/kg body weight. The third group was injected with recombinant bovine interferon gamma (rbIFN-γ) in a dose of 10^6 U/kg (50 μg/kg) body weight. The doses of rbTNF-α and rbIFN-γ were used based on our previous study (Soliman et al. 2004). The highly purified rbIFN-γ was provided by Dr. Shigeki Inumaru, National Institute of Animal Health, Tsukuba, Japan, produced by a baculovirus gene expression system. The rbTNF-α was provided by Higeta Shoyu Co., Ltd., Choshi, Japan. The rbTNF-α was produced by a Bacillus brevis host-vector system. Blood was collected from the jugular vein of all experimental animals as described in Soliman et al., 2004; serum was separated by centrifugation at 5000 rpm for 10 min and stored at -20 °C until measurement of serum haptoglobin. Results reported are limited to the sample collected 48 h after the onset of injections based on the significant increase of Hp observed therein.

Haptoglobin measurements

An Hp assay kit was purchased from Tridelta Development Limited, Greystones Co. Wicklow. Ireland. Hp was measured according to manufacturer's instruction manual. Briefly, the assay principally depends on the fact that Hp present in specimen can combine with hemoglobin and at low pH preserves the peroxidase activity of bound hemoglobin. Preservation of peroxidase activity of hemoglobin is directly proportional to

the amount of Hp in serum samples. Each sample was assayed in triplicates. The inter- and intra-assay coefficient of variation was around 5%. Based on the kits instructions used in this study (PHASE"™ Haptoglobin Assay Cat. No. TP-801**),** the normal range of serum Hp concentrations in cattle are below 0.05 mg/ml in cattle and may increase up to 1000-fold in response to acute infection, inflammation or trauma.

Tissue sampling and isolation of stromal vascular cells from subcutaneous adipose tissue

Three non-pregnant, non-lactating Holstein cows (2-3 years old) were slaughtered in the college slaughter house. Samples from the mammary gland, kidney, spleen, rumen wall, small intestine, subcutaneous and pararenal adipose tissue, liver, skeletal muscle (biceps), pancreas, hypothalamus, medulla oblongata, heart, adrenal gland, and lung were collected under sterile conditions and kept -80 ^{0}C in TriZol reagent for total RNA extraction and qualitative RT-PCR analysis. For isolating the stromal vascular cell fraction containing preadipocytes, specimen from subcutaneous adipose tissue (100-500 mg) of the neck were dissected into small pieces (100 μm in size) and transferred into Hank's balanced salt solution containing 2 mg/mL collagenase and 0.1% BSA (in a sterile 50 mL plastic tube). Digestion of specimen was carried out at 37 °C for 90 min with gentle shaking. The cells were then filtered through a sterile nylon mesh with 80 μm pores. The filtrate was centrifuged at 1000 x *g* for 5 min at room temperature. The collected cells were treated with an erythrocyte lysis buffer (154 mM NH_4CL, 10 mM $KHCO_3$, 1 mM EDTA) for 5 min and washed twice with DMEM containing 10% FCS, 100 U/mL penicillin and 100 μg/mL streptomycin. The cells were cultured in DMEM (5.5 mM glucose) containing 10% FCS on collagen-coated dishes (Wako Pure Chemicals Co. (Osaka, Japan). Cultured cells were incubated at 37 °C and 5% CO_2 and the media were changed every 2 days. The cells between 3rd and 5th passage were used for the experiments.

Differentiation of preadipocytes to mature adipocytes and stimulation experiments

Differentiation of bovine preadipocytes was based on the protocol described by Soliman et al. (2007). Briefly, when the stromal vascular cells had reached 95% confluence (referred to as day 0), the cells were cultured in DMEM/F12 containing 1.5% FCS, 0.5 mM IBMX, 1 µM dexamethasone, and 10 µg/ml insulin for 2 days. After initial 2 days of differentiation, cells were maintained in fresh DMEM/F12 containing 1.5% FCS, 10 µM troglitazone and 10 µg/ml insulin. Media were changed every 2 days for 12 days to achieve full cell differentiation and maturation. At day 14, the cells were cultured in DMEM without glucose and FCS, but containing 0.1% BSA for 24 h and further cultured in DMEM with 2.5 mM glucose containing 0.1% BSA (control) in the absence or presence of different regulators. Cells were incubated with each 1 mM of short chain fatty acids, i.e. acetate (Ac), butyrate (Buty) or propionate (Prop) for 48 h. In addition, cells were treated with LPS, TNF, estradiol-17ß, norepinephrine and troglitazone for 48 h, and then RNA was thereafter extracted for RT-PCR analysis. All *in vitro* experiments were carried with adipocytes derived from 3 different cows and were run in triplicate for each cow.

RNA extraction, cDNA synthesis and RT-PCR analysis

Total RNA was isolated from various organs, tissues and from differentiated mature adipocytes by the guanidine-isothiocyanate method using TRIzol reagent (Gibco BRL, Rochville, MD, USA). The RNA integrity was checked by electrophoresis; RNA concentration and purity were determined spectrophotometrically at 260 nm. The ratio of the 260/280 optical density of all RNA samples was 1.7-1.9. Total RNA (1 µg) was denatured at 72 °C for 5 min and reverse transcribed using 100 units of Moloney Murine Leukaemia virus reverse transcriptase (Gibco), 50 pmol of poly (dT) primer and 20 nmol of dNTPs in a total volume of 10 µl at 37 °C for 1 h. After heating at 94 °C for 5 min, PCR amplification was performed with 2.5 units Taq polymerase (Perkin-Elmer, Foster City, CA, USA), 3 mM MgCl2 and 50 pmol of forward and reverse primers specific for respective genes in a total volume of 25 µl. The primer pairs and PCR condition of bovine Hp was as follows: forward is 5′-GTCTCCCAGCATAACCTCAT-3′ and reverse is 5′-TGGGCATTACTTTGTCATTG-3' (Gene Bank NM_001040470), annealing temperature was 58 ºC for 1 min, 30 cycles were run and the amplified Hp PCR product

size was 242 bp. Bovine glyceraldehydes-3-phosphate dehydrogenase (G3PDH) was used as internal standard (Gene Bank U85042). Primer used for G3PDH was 5'-ACCACTGTCCACGCCATCAC-3' for forward primer and 5'-TCCACCACCCTGTTTGCTGTA-3' for reverse primer. The PCR reaction was carried out for 25 cycles and at an annealing temperature of 59 ºC for 30 sec to amplify a product of 450 bp size. PCR products were visualized after electrophoresis in 1.5% agarose gel stained with ethidium bromide. The mRNA of bovine Hp and G3PDH amplified from total RNA of differentiated bovine adipocytes was subcloned into pGEM-T Easy vector (Promega; Madison, WI, U.S.A). The nucleotide sequence of each cDNA was confirmed using Bio-Rad DNA sequencer and the cDNAs were used as probes for Northern blot analysis.

Northern blot analysis

Total RNA (20 μg) was resolved on 1% agarose-formaldehyde gel, transferred onto a nylon membrane (Hybond-N+; Amersham Pharmaceutical Biotec. Buckinghamshire, UK) and cross-linked under UV light for 2 min. Both prehybridization and hybridization were performed at 65□C for 2 h and overnight, respectively, in a buffer containing 7% SDS, 0.5 M Church's phosphate buffer, pH 7.2, 1 mM EDTA and 0.5 mg/ml salmon sperm DNA. After prehybridization, the membrane was sequentially hybridized with a cDNA probe encoding bovine Hp and bovine G3PDH as the internal control for loading. The probe was labeled with [α–^{32}P] dCTP using the Megaprime^{TM} DNA labeling systems (Amersham) according to the instructions provided. After hybridization, the membrane was stringently washed for 20 min twice with 2x SSC and 0.1% SDS, and once with 0.1 x SSC and 0.1% SDS at 65 °C before exposure onto a phospho-imaging plate overnight. Detection and quantification of the hybridization signals were carried out using a phosphoimage analyzer (BAS 2500, FUJIFILM, Tokyo, Japan) after normalization to G3PDH signaling intensity. Each experiment was done in triplicate.

Statistical analysis

Data for serum Hp are expressed as means ± standard error of 3 different cows per group (*in vivo* experiment), and as means ± standard error of means for 3 independent experiments for each treatment (*in vitro* experiment). Statistical analysis for both experiments (*in vivo* and *in vitro*) was done using ANOVA and Fischer's post-hoc test, with $P < 0.05$ being considered as statistically significant.

Results

Expression of the haptoglobin mRNA in different bovine tissues

Tissue distribution of Hp was examined in various tissues using qualitative RT-PCR analysis. Figure 1a shows that Hp was not only expressed in liver but also in other tissues. Positive signals for Hp mRNA were obtained in most tissues except spleen probably the RNA quality is the cause.

Expression of haptoglobin mRNA during differentiation of bovine preadipocytes

During the first 4 days of cell differentiation Hp mRNA expression was not detectable. When cells became mature, the expression increased and was time dependently reaching a maximum at day 14 (Figure 1 b).

Effect of rbTNF-α and rbIFN-γ on the concentrations of Hp in serum

Intravenous injection of rbTNF-α and rbIFN-γ induced an increase in Hp serum concentrations in dairy cows, reaching significant higher values more than in saline treated control cows 48 h after the injection. At that time, as shown in Figure 2, the TNF-α injection induced a 3-fold increase while IFN-γ induced approximately a 2-fold increase in Hp concentrations, respectively.

Effect of different short chain fatty acids and of other potential regulators on Hp mRNA expression in differentiated bovine adipocytes

Incubation of differentiated mature adipocytes with each 1 mM acetate (Ac), butyrate (Buty) or propionate (Prop) for 48 h increased Hp mRNA abundance (Figure 3). When bovine adipocytes were incubated for 48 h with different doses of LPS, TNF-α, Estradiol-

17ß, norepinephrine (NE), troglitazone, dexamethasone or trijodthyronine (T3), Hp expression was mostly increased. As shown in Figure 4 a, LPS and NE stimulated Hp mRNA expression at all dosages tested. Significant increases of Hp mRNA expression were also observed at 10 and 50 but not 5 ng/ml of TNF-α, as well as at 10 and 100 but not 10 nM Estradiol-17ß. Dexamethasone at 100 but not 10 nM stimulated Hp mRNA expression, and T3 at 50 but not 1 nM increased the Hp mRNA abundance above the controls (Figure 4 b). In contrast, troglitazone as well as insulin reduced dose dependently Hp mRNA expression (Figure 4 a and b).

Discussion

The present findings confirm that bovine Hp as an acute phase protein is not only expressed in liver but also in various other tissues of cattle. Interpretation of all semiquantiative results from the stimulation experiments with *in vitro* differentiated bovine adipocytes obtained by Northern blotting is based on the assumption that the expression of G3PDH used as reference remained constant across all treatments. Hp expression in differentiated bovine adipocytes was demonstrated to be regulated by short chain fatty acids (nutritional control) and other factors. The expression of Hp in adipose tissue and after in adipocytes cell maturation suggests that Hp might be a marker for differentiation as reported in 3T3L-1 cells (do Nascimento et al., 2004 and Friedrichs et al., 1995) and in bovine adipocytes (Saremi et al., 2010). As known, Hp synthesis in bovine hepatocytes is regulated by different mechanisms from those reported in rodents and humans (Yoshioka et al. 2002). TNF-α stimulates Hp synthesis in bovine hepatocytes *in vitro* (Nakagawa-Tosa et al., 1994).

The mechanism by which rbTNF and rbIFN affect Hp secretion is still unclear but cyclooxygenase-2 activity may be involved (Fain et al., 2004). Soliman et al. (2004) reported that rbTNF-α and rbIFN-γ modulated adrenocortical activity and increased the serum concentrations of cortisol, glucose, and several cytokines in cattle indicating increasing metabolic activity. The increase in Hp mRNA by dexamethasone we observed herein is in line with the report of Lyoumi et al. (1999). Similar stimulatory effects of dexamethasone have been reported for other adipokines such as leptin, metallothionein, and resistin (Trayhurn and Beattie 2001). Dexamethasone stimulated Hp expression in

bovine adipocytes as well as in 3T3L-1 cells (Nakagawa-Tosa et al. 1994 and Yoshioka et al. 2002), but in human adipose tissue explants dexamethasone decreased Hp production (Fain et al. 2004). The contrariness of these results may be explained by other factors in tissue explants that inhibited the direct *in vitro* stimulatory effect reported in our results and that is supported by the results of do Nascimento et al. (2004).

Fatty acids might affect on Hp expression and/or secretion. For example, it has been shown that dietary supplementation with conjugated linoleic acids (CLA) has no effect on the circulating Hp concentrations, on the Hp mRNA abundance in liver and in most adipose tissue depots (Saremi et al. 2012), while our findings showed the up-regulation of Hp mRNA expression by short chain fatty acids. This suggests fatty acid-specific effects on Hp secretion and expression; besides differences in the *in vivo* and *in vitro* methodology used in both studies might account for the divergent effects. We have previously shown that the difference in the number of carbon atoms in fatty acids affected the response of bovine adipocytes with regard to leptin expression (Soliman et al., 2007). Fatty acids are nutritients that play a number of key roles in metabolic regulation. They act as ligands for transcription factors (Drevon et al. 2005; Rusta el al. 2003). Moreover, diet rich in lipids may provide the body with polyunsaturated fatty acids (PUFA) that are rich in eicosanoids (powerful locally acting metabolites; Drevon et al. 2005). These fatty acids and their derivatives probably interact with nuclear receptor proteins and alter the transcription of some target genes (Drevon et al. 2005).

For estradiol-17ß, we observed stimulatory effects on Hp mRNA expression, however, the biological importance of this finding remains open since the dosages tested as effective were well above the physiological range of serum concentrations typical for cyclic female cattle. However, when comparing peri-estrous with luteal cows, hepatic Hp mRNA expression was found to be elevated above basal (Lavery et al., 2004). More studies are needed to outline the functional importance of estrogens for Hp expression. In cultured bovine adipocytes, LPS and TNF stimulated Hp mRNA expression while troglitazone and insulin inhibited it. It is worth noting that Hp is expressed and secreted from adipose tissue (do Nascimento et al., 2004) and up-regulated in blood and adipose tissue of obese but not lean human subjects (Soukas et al., 2000; Chiellini et al., 2002). As known, adipose tissue acts as an endocrine organ that secretes cytokines and proteins

that are involved in the regulation of body defensive mechanism, health status and reproductive events (Mohamed-Ali et al. 1998; Lavery et al. 2004). Inflammation and obesity share the modulation of various cytokines including IL-6 and TNF-α (Mohamed-Ali et al., 1998; Sethi and Hotamisligil 1999). It has been reported that hepatic expression of Hp is regulated in rodents by IL-1, IL-6, glucocorticoids and TNFα, while by IL-6 and glucocorticoids in humans (Baumann et al., 1990; Mackiewicz et al., 1991). LPS up-regulates Hp expression in bovine adipocytes (Saremi et al, 2012). Our results confirmed the involvement of LPS, TNF-α and some hormones in regulation of Hp expression in bovine adipocytes. In other species, TNF-α was reported to be the common inflammatory cytokine mediating Hp gene expression (Pajovic et al. 1994); in monogastric species, TNF-α is increased with adiposity, however, no such reports exist for cattle. TNF-α blocks PPARγ but troglitazone is a stimulator of both PPARα and PPARγ. The catecholamine NE stimulated Hp mRNA expression in bovine adipocytes in our study, and thus suggests the involvement of ß-adrenergic receptors. The expression of the three main subtypes of ß-adrenergic receptors ß1, ß2 and ß3 has been demonstrated in bovine adipose tissue (Sumner and McNamara, 2007). Regarding T3 effects on Hp expression, it has been shown that T3 administration in rats induces the acute-phase response and haptoglobin in rat liver by a redox mechanism triggered at the Kupffer cell levels (Tapia et al., 2006) and in hepatocellular carcinoma cell lines (Li et al., 2003). This *in vivo* increase in Hp is in line with the *in vitro* findings reported in this study.

Conclusion

This study extends the number of tissues in which haptoglobin mRNA is expressed in cattle. The observation that the short chain fatty acids acetate, propionate and butyrate were able to stimulate Hp mRNA expression in bovine adipocytes differentiated *in vitro*, indicates that a direct regulation by nutrients may occur. Moreover, the involvement of metabolic hormones such as glucocorticoids, catecholamines, insulin and thyroid hormones in regulating Hp mRNA expression besides “classical” proinflammatory stimuli points to metabolic signals affecting the transcription of this particular acute phase protein in differentiated adipocytes. Further studies are needed to address the

functional relevance of the metabolically-induced alteration of Hp mRNA expression in adipocytes.

Competing interests

The authors declare that there is no competing interest.

Authors' contributions

MMS carried out experimental design, MMS and TAI carried all experimental techniques. MMS and AAM carried out statistical analysis.

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References

Arredouani, M., P. Matthijs, E. Van Hoeyveld, A. Kasran, H. Baumann, L. Ceuppens, and E. Stevens. 2003. Haptoglobin directly affects T cells and suppresses T helper cell type 2 cytokine release. Immunol 2: 144-151.

Baumann, H., K.K. Morella, G.P. Jahreis, and S. Marinkovic. 1990. Distinct regulation of the interleukin-1 and interleukin-6 response elements of the rat haptoglobin gene in rat and human hepatoma cells. Mol Cell Biol 10: 5967-5976.

Bertaggia, E., Scabia, G., Dalise, S., Lo Verso, F., Santini, F., Vitti, P., Chisari, C., Sandri, M, and M. Maffei. 2014. Haptoglobin is required to prevent oxidative stress and muscle atrophy. PLoS One 9 (6):e100745.

Chiellini, C., A. Bertacca, E.S. Novelli, Gorgun, Z.C, A. Ciccarone, A. Giordano, H. Xu, A. Soukas, M. Costa, D. Giandini, R. Dimitri, P. Bottone, P. Cecchetti, E. Pardini, L. Perego, R. Navalesi, F. Folli, L. Benzi, S. Cinti, M.J. Friedman, S.G. Hotamiligil, and M. Maffei. 2002. Obesity modulates the expression of haptoglobin in the white adipose tissue via TNFα. J Cell Physiol 190: 251-258.

Dilda, F., L.F. Pisani, M.M. Rahman, S. Modina, I. Tessaro, P. Sartorelli, F. Ceciliani, and C. Lecchi. 2011. Distribution of acute phase proteins in the bovine forestomachs and abomasum. Vet J 192(1): 101-105.

de Kleijn, D.P., M.B. Smeets, P. P. Kemmeren, S.K. Lim, B. J. Van Middelaar, E. Velema, A. Schoneveld, G. Pasterkamp, and C. Borst. 2002. Acute-phase protein haptoglobin is a cell migration factor involved in arterial restructuring. FASEB J 9:1123-1125.

do Nascimento, C.O., L. Hunter, and P. Trayhurn. 2004. Regulation of haptoglobin gene expression in 3T3-L1 adipocytes by cytokines, catecholamines, and PPAR-γ. Biochem Biophys Res Commun 313: 702–708

Doumatey, A.P., K.S Lashley, H. Huang, J. Zhou, G. Chen, A. Amoah, K. Agyenim-Boateng, J. Oli, O. Fasanmade, C.A. Adebamowo, A.A. Adeyemo, and C.N. Rotimi. 2009. Relationships among obesity, inflammation, and insulin resistance in African Americans and West Africans. Obesity 18, 598–603.

Drevon, C. A. 2005. Fatty acids and expression of adipokines. Biochimica et Biophysica Acta 1740 (2):287-92.

Eckersall, P.D and R. Bell. 2010. Acute phase proteins: biomarkers of infection and inflammation in veterinary medicine. Veterinary Journal 185: 23–27.

Fain, N., J, Suleiman, W. Bahouth, and K. Madan Atul. 2004. Haptoglobin release by human adipose tissue in primary culture. J Lipid Res 45: 536–542.

Friedrichs, W.E., A.L. B. Navarijo-Ashbaugh, H. Bowman, and F. Yang. 1995. Expression and inflammatory regulation of haptoglobin gene in adipocytes. Biochem Biophys Res Commun 209: 250-256.

Godson, D.L., M. Campos, S.K. Attah-Poku, M.J. Redmond, D.M .Cordeiro, M.S. Sethi, R.J. Harland, and L.A. Babiuk. 1996. Serum haptoglobin as an indicator of the

acute phase response in bovine respiratory disease. Vet Immunol Immunopathol 51: 277–292.

Heinrich, P., J.V. Castell, and T. Andus. 1990. Interleukin-6 and the acute phase response. Biochem J 265: 621-636.

Huntoon, K.M., Y. Wang, C.A. Eppolito, K.W. Barbour, F.G. Berger, P.A. Shrikant, and H. Baumann. 2008. The acute phase protein haptoglobin regulates host immunity. J Leukoc Biol 84:170-181.

Lavery, K., C. Gabler, J. Day, and G. Killian. 2004. Expression of haptoglobin mRNA in the liver and oviduct during the oestrous cycle of cows (Bos taurus). Anim Reprod Sci 84: 13–26.

Lyoumi, S., H. Puy, F. Tamion, C. Bogard, A. Leplingard, M. Scotte, R. Vranckx, F. Gauthier, M. Hiron, M. Daveau, Y. Nordmann, J.C. Deybach, and J.P. Lebreton. 1999. Heme and acute inflammation role in vivo of heme in the hepatic expression of positive acute-phase reactants in rats. Eur J Biochem 261: 190-196.

Mackiewicz, A., T. Spero, M.K. Ganapathi, and I. Kushner. 1991. Effects of cytokine combinations on acute phase protein production in two human hepatoma cell lines. J Immunol 146: 3032–3037.

Mohamed-Ali, V., J.H. Pinkney, and S.W. Coppack. 1998. Adipose tissue as an endocrine and paracrine organ. Int J Obes Relat Metab Disord 22: 1145–1158.

Nakagawa-Tosa, N., M. Morimatsu, M. Kawasaki, H. Nakatsuji, B. Syuto, and M. Saito. 1994. Stimulation of haptoglobin synthesis by interleukin-6 and tumor necrosis factor, but not by interleukin-1, in bovine primary cultured hepatocytes. J Vet Med Sci 57: 219–223.

Pajovic, S., V.E. Jones, K.R.Prowse, F.G. Berger, and H. Baumann. 1994. Species-specific changes in regulatory elements of mouse haptoglobin genes. J Biol Chem 269: 2215–2224.

Putnam, F. 1975. Haptoglobin. In The Plasma Proteins: Structure, Function, and Genetic Control. 2nd ed. Vol. 2. F. W. Putnam, editor. Academic Press, New York, 1–51.

Quaye, K. I. 2008. Haptoglobin, inflammation and disease. Trans Royal Soc Trop Med Hyg 102:735-742.

Rusta, A.C., and C.A. Drevon. 2000. Fatty Acids: Structures and Properties. Encyclopedia of Life Sciences, Nature Publishing, London. http://www.els.net.

Saremi, B., A. Al-Dawood, S. Winand, U. Müller, J. Pappritz, D. von Soosten, J. Rehage, S. Dänicke, S. Häussler, M. Mielenz, and H. Sauerwein. 2012. Bovine haptoglobin as an adipokine: serum concentrations and tissue expression in dairy cows receiving a conjugated linoleic acids supplement throughout lactation. Vet Immunol Immunopathol 146 (3-4):201-211.

Saremi, B., M. Mielenz, A. Hosseini, C. Behrendt, S. Vorspohl and H. Sauerwein. 2010. Haptoglobin expression in different adipose tissues and adipocytes (*in vitro*) of dairy cows. Proc 19th Symp of the German Veterinary Society, Section Physiology and Biochemistry, Hannover; 2010. p. 25. ISBN: 978-3-941703-551.

Sethi, J.K. and G.S. Hotamisligil. 1999. The role of TNFα in adipocyte metabolism. Semi cell Dev Biol 10: 19-29.

Skinner, J.G. and L. Roberts. 1994. Haptoglobin as an indicator of infection in sheep. Vet Rec 134: 33-36.

Soliman, M., K. Kimura, M. Ahmed, D. Yamaji, Y. Matsushita, Y. Okamatsu-Ogura, K. Makondo, and M. Saito. 2007. Inverse regulation of leptin mRNA expression by short- and long-chain fatty acids in cultured bovine adipocytes. Dom Anim Endocrinol 33(4): 400-409.

Soliman, M., S. Okamoto, H. Kitamura, S. Kushibiki, K. Kimura, and M. Saito. 2004. Adrenocortical responses to tumor necrosis factor-α and interferon-gamma in cattle. Jpn J Vet Res 52 (2): 95-99.

Soukas, A., P. Cohen, N.D. Socci, and J.M. Friedman. 2000. Leptin-specific patterns of gene expression in white adipose tissue. Genes Dev14, 963-980.

Sumner JM[1], McNamara JP. 2007. Expression of lipolytic genes in the adipose tissue of pregnant and lactating Holstein dairy cattle. J Dairy Sci 90(11):5237-5246.

Tapia, G., Fernández, V., Pino, C., Ardiles, R. and Videla LA. 2006. The acute-phase response of the liver in relation to thyroid hormone-induced redox signaling. Free Radic Biol Med40(9):1628-35.

Thielen, M.A., M. Mielenz., S. Hiss, and H. Sauerwein. 2005. Qualitative detection of haptoglobin mRNA in bovine and human blood leukocytes and bovine milk somatic cells. Vet Med 50: 515–520.

Thielen, M.A., M, Mielenz, S. Hiss, H. Zerbe, W. Petzl, H.J. Schuberth, H.M. Seyfert, and H. Sauerwein. 2007. Short communication: cellular localization of haptoglobin mRNA in the experimentally infected bovine mammary gland. J Dairy Sci 90: 1215–1219.

Trayhurn, P.J. and H. Beattie. 2001. Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. Proc Nut Soc 60: 329-339.

Yoshioka, M., A. Watanabe, N. Shimada, H. Murata, Y. Yokomizo, and Y. Nakajima. 2002. Regulation of haptoglobin secretion by recombinant bovine cytokines in primary cultured bovine hepatocytes. Dom Anim Endocrinol 23: 425–433.

Zarrin, M., Wellnitz, O., van Dorland, H.A, and R.M. Bruckmaier. 2014. Induced hyperketonemia affects the mammary immune response during lipopolysaccharide challenge in dairy cows. J Dairy Sci 97(1):330-339.

Figure Legends

Fig.1. Tissue distribution of Hp in cattle tissues (A) and during differentiation of bovine adipocytes (B) using qualitative RT-PCR analysis in A and Northern blot analysis in B. In A, total RNA (1 µg) was extracted and reverse transcribed and visualized in 1.5 % agarose gel stained with ethidium bromide. Bands are 1; mammary gland; 2, kidney; 3, spleen; 4, rumen wall; 5, small intestine; 6, subcutaneous adipose tissue;7, liver; 8, skeletal muscle (Biceps); 9, pancreas; 10, para-renal AT; 11, hypothalamus; 12, heart; 13, testis; 14, adrenal gland; 15, lung and 16 is medulla oblongata. In B, total RNA was extracted from day 0 to day 14 during cell differentiation and subjected to Northern blot analysis as described in materials and methods to examine Hp expression. In B; Hp expression was denistometrically analyzed relative to G3PDH and compared to day 0. Data are means ± SEM for 3 independent experiments. *P< 0.05 compared to day 0.

Fig. 2. Effect of rbTNF-α and rbIFN-γ on serum Hp levels in Holstein cows. 3 cows were injected intravenously either PBS as control, or rbTNF-α 5µg/kg, or rbIFN-γ (10^6U/kg). Blood sampling after 48 h was carried. Serum was extracted and Hp was measured using

commercial kits. Values are means ± SEM for 3 independent experiments. *P< 0.05 compared to control.

Fig. 3. Short chain fatty acids up-regulate Hp expression in mature bovine adipocytes. Differentiated bovine adipocytes were incubated with 1 mM acetate (Ac), butyrate (Buty) and propionate (Prop) for 48 h. Total RNA was extracted and subjected to Northern blot analysis as described in materials and methods. Data and densitometry are represented as means ± SEM for 3 independent experiments. *P< 0.05 compared to control.

Fig.4. Effect of some regulators on Hp expression in bovine adipocytes. In A, Cells were taken at day 14 of differentiation and incubated for 48 h in media containing: insulin (Ins,1 nM, Ins L; 100nM, Ins H), LPS (25 ng/ml; 50ng/ml; 100 ng/ml), TNF-alpha (TNF-α, 5 ng/ml; 10 ng/ml; 50 ng/ml), estradiol 17β (E2, 10 nM; 50 nM;100 nM), nor-epinephrine (NE, 1μM; 10 μM) and troglitazone (Trog) in dose of 1μM and 10 μM. In B, cells were incubated with variable doses of insulin (1 nM, Ins L; 100 nM, Ins H), dexamethasone (1 nM, Dex L; 100 nM, Dex H), Norepinephrine (1 μM, NE L; 10 μM, NE H), estrogen (10 nM, Est L; 100nM Est H), tri-iodothyronine (1 nM, T3 L; 50 nM, Est H). Total RNA (20 μg) was extracted from cells and subjected to Northern blot analysis as described in materials and methods. Data and densitometry are represented as means ± SEM for 3 independent experiments. *P< 0.05 compared to control. $^{\#}$P<0.05 compared to troglitazone at 1 μM.

Figure 1

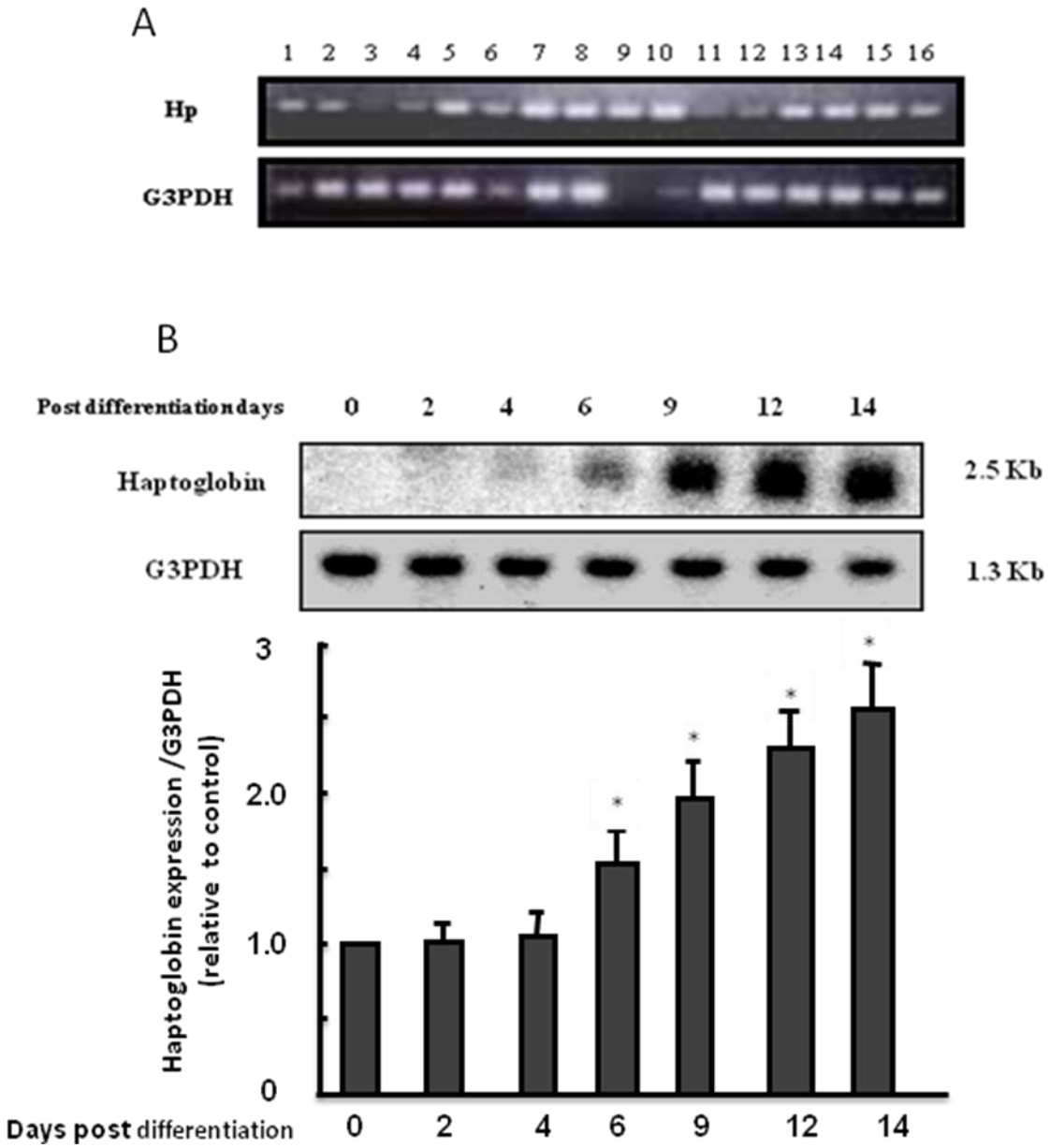

Figure 2

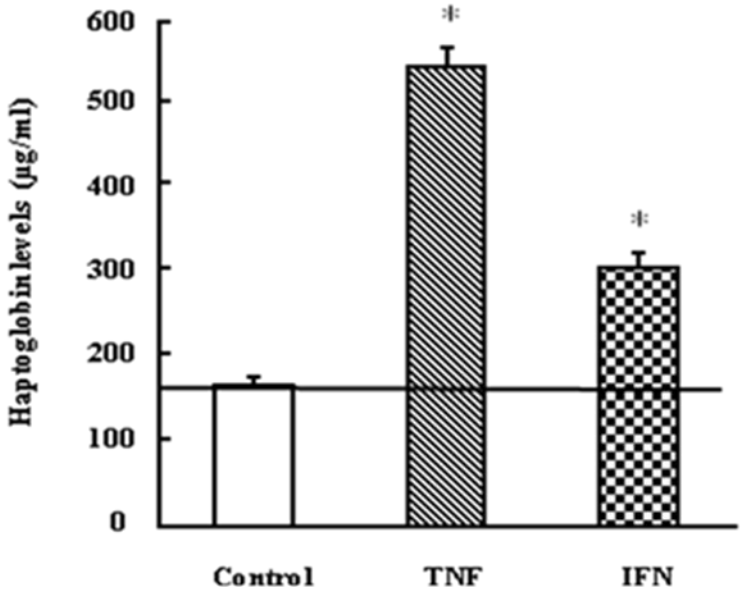

Figure 3

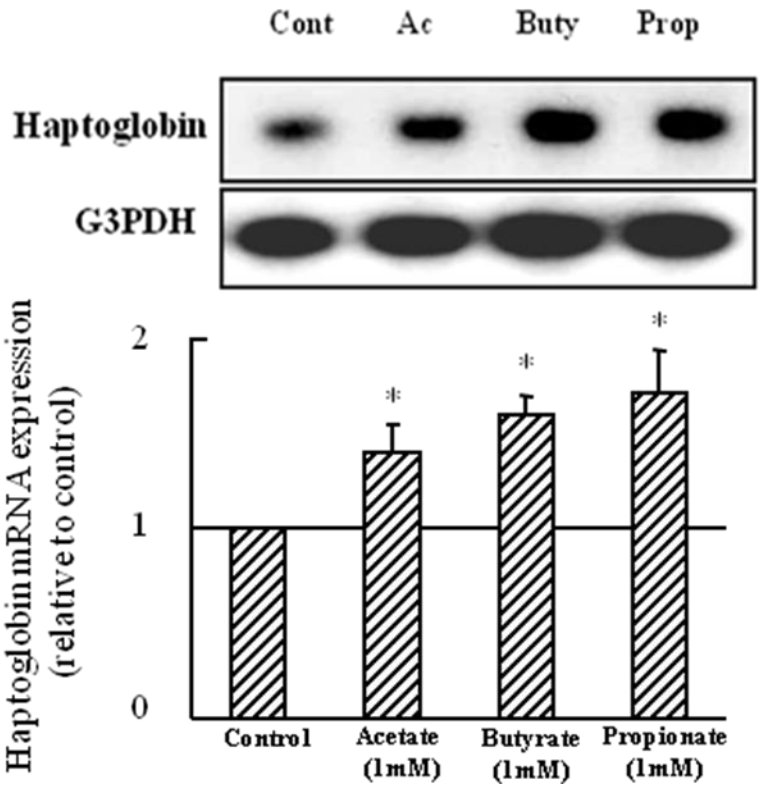

Figure 4