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Distinct glycoforms of human α_1-acid glycoprotein have comparable synthesis rates: a [^{13}C]valine-labelling study in healthy humans

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Various α_1-acid glycoprotein (AGP) glycoforms are present in plasma differing in extent of branching and/or fucosylation of their 5 N-linked glycans, as well as in concentration. It is assumed that hepatic synthesis determines the relative occurrence of the AGP-glycoforms in plasma, but experimental evidence is lacking. In this study, we have investigated the contribution of fractional synthesis rates to the plasma concentration of AGP-glycoforms that differed in relative occurrence in healthy human plasma. During a [^{13}C]valine infusion, AGP was isolated from the plasma of healthy volunteers. Four AGP-glycoforms, differing strongly in plasma concentration were obtained by sequential affinity chromatography over concanavalin-A- and *Aleuria aurantia*-agarose columns. The incorporation of the [^{13}C]valine tracer into the AGP-glycoforms was measured by gas chromatography combustion isotope ratio mass spectrometry. The mean fractional synthesis rates of the four AGP-glycoforms did not differ significantly between each other as well between individuals. The results indicated a renewal of about 15%/day of the plasma pools of each of the AGP-glycoforms. This is in support to the assumption that the differences in plasma concentration of the AGP-glycoforms are a reflection of the state of the hepatic glycosylation process. ***Published in 2004.***

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Abbreviations: **AAL, *Aleuria aurantia* lectin; AGP, α_1-acid glycoprotein; Con A, concanavalin A; FSR, fractional synthesis rate; mab-AGP, monoclonal anti-AGP IgG; TTR, tracer-to-tracee molar ratio; GC-c-IRMS, gas chromatography combustion isotope ratio mass spectrometry.**

Introduction

α_1-Acid glycoprotein (AGP) is a human plasma protein that belongs to the group of positive acute-phase proteins, which are produced by the liver [1,2]. It has a carbohydrate content of 45% in the form of 5 N-linked glycans. These glycans display a microheterogeneity with respect to their extent of branching (di-, tri- and tetraantennary glycans), degree of α3-fucosylation and sialylation [3–5]. This is reflected in the presence of various AGP-glycoforms in plasma [6–8]. The concentrations and relative occurrence of AGP-glycoforms reactive with concanavalin A (Con A; recognizing di-antennary-containing AGP-glycoforms) or *Aleuria aurantia* lectin (AAL; recognizing α3-fucosylated AGP-glycoforms) have been shown to fall between narrow limits in a healthy population of human individuals [9]. Temporal changes in plasma concentrations of specific AGP-glycoforms occur during hepatic acute-phase reactions like induced by severe trauma. This regards Con A-reactive AGP-glycoforms with two or more di-antennary glycans as well as strongly fucosylated AAL-reactive AGP-glycoforms expressing sialyl Lewis^x groups [7,8,10–12]. The physiological function of AGP is still in debate, since it can bind and carry numerous basic and neutral lipophilic compounds [13,14], but also expresses several anti-inflammatory properties [1,8,9]. Studies *in vitro* as well as in animals have

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shown that the anti-inflammatory properties are strongly influenced by the composition of its glycans, i.e. are AGP-glycoform dependent [8,15–17]. For instance, inhibition of CD3-induced proliferation of lymphocytes by AGP requires the presence of diantennary glycans [18], and amelioration of neutrophil- and complement-mediated injuries can be performed by AGP-glycoforms that contain sialyl Lewis^X groups [17].

We and others have anticipated that increases in plasma concentration of specific AGP-glycoforms are induced by hepatic synthesis and will contribute to the beneficial effects of the hepatic acute-phase reaction [7,8,17]. Indeed, inflammatory mediators implicated in the regulation of the hepatic acute-phase reaction have been shown to induce the synthesis of diantennary-containing AGP-glycoforms by human hepatocytes *in vitro* [16], most probably by changing the expression of specific glycosyltransferases [6,19]. The relative increase of these AGP-glycoforms in the secretion media corresponded to the changes observed in the acute-phase induced increases observed in human plasma for these AGP-glycoforms. This suggests that in human plasma the relative abundance of AGP-glycoforms is a synthesis-driven phenomenon, but experimental evidence is lacking. Therefore, we have performed a [^{13}C]valine-labelling study of AGP-glycoforms in healthy human subjects to determine the fractional synthesis rates (FSR) of four AGP-glycoforms that differ strongly in plasma concentration.

Methods

Human study protocol

Five healthy, non-smoking male volunteers with normal plasma levels (0.5–1.1 g/L) of AGP (see Table 1 for subject characteristics) were recruited. The study protocol was approved by the local ethics committee, and informed consent was obtained from each subject. L-[1-^{13}C]Valine (99% ^{13}C) was prepared as sterile solution. After overnight fasting the patients reported at 8.00 h, and Teflon catheters were inserted into a dorsal hand vein of each hand. A primed (7.0 mmol $\cdot$ kg^{-1}), continuous infusion of L-[1-^{13}C]valine (7.0 mmol $\cdot$ kg^{-1} $\cdot$ h^{-1}) was started ($t = 0$) and continued during 6 h. Arterialized blood samples were obtained with a heated hand box [20]. The blood samples were drawn at $t = 0$ for background enrichments, and at 120, 180, 240, 300 and 360 min for precursor enrichments and AGP-glycoform isolations. Heparinized blood samples were placed on ice, centrifuged at 4°C, and stored at −70°C until analysis.

Materials

Concanavalin A (Con A) (Type V), Coomassie Brillant Blue R250, L-fucose and α-methylmannopyranoside were from Sigma (ST. Louis, MO, USA). *Aleuria aurantia* lectin (AAL) and the AAL-HiTrap columns were obtained from Biomed Labs (Newcastle upon Tyne, UK). Both the FPLC system, the 5 ml HiTrap desalting columns, the 1-ml affinity HiTrap columns and the DEAE-Sepharose were obtained from Pharmacia (Uppsala, Sweden), polyacrylamide and agarose M from BioRad (Richmond, CA, USA) and human serum protein calibrator (HSPC) and monospecific rabbit-antihuman AGP antiserum from Dakopatts (Glostrup, Denmark). 1-ml Con A-HiTrap and mab-AGP-HiTrap columns were prepared according to the procedure provided by the manufacturer. Monoclonal anti-AGP IgG (mab-IgG) was a gift of Dr. B. Halsall (Cincinnati, Ohio, USA). L-[1-^{13}C]Valine (99% ^{13}C) was obtained from Mass Trace (Woburn, MA, USA).

Isolation of total AGP

AGP was isolated from 5-ml plasma by FPLC affinity chromatography (see Figure 1A). Binding of AGP, on three combined 1-ml mab-AGP HiTrap columns containing 6 mg mab-AGP (IgG_3) each, was performed in PBS. Bound AGP was eluted with PBS containing 1.15 M NaCl and desalted in demineralized and filtered water (milli-Q water) by two 5-ml desalting HiTrap columns. The samples were lyophilized, dissolved in milli-Q water, followed by concentration determination and checked for purity on 10% SDS-PAGE. The samples were stored at −20°C until further analysis.

Isolation of different AGP-glycoforms

FPLC affinity chromatography with the lectins, Con A and AAL as affinity component, was applied to separate the different glycoforms of AGP according to their diantennary glycan presence and degree of fucosylation, respectively [8]. Four combined 1-ml Con A-HiTrap columns containing 10 mg Con

Table 1. Characteristics of selected male human volunteers

Subject	*Age (years)*	*BMI* (kg/m^2)*	*CRP** (mg/L)*	*Glucose (mM)*	*Albumin (g/L)*	*AGP concn. (mg/L)****
1	23	20.8	5.0	4.7	41	690 ± 80
2	21	25.3	4.0	4.8	39	990 ± 40
3	27	18.9	3.0	5.0	40	980 ± 70
4	22	20.7	<2.5	5.1	44	520 ± 90
5	27	21.1	<2.5	5	40	930 ± 60

*BMI, body mass index.
**CRP, C-reactive protein.
***Mean ± S.D. of the values as determined at the various time points ($n = 6$).

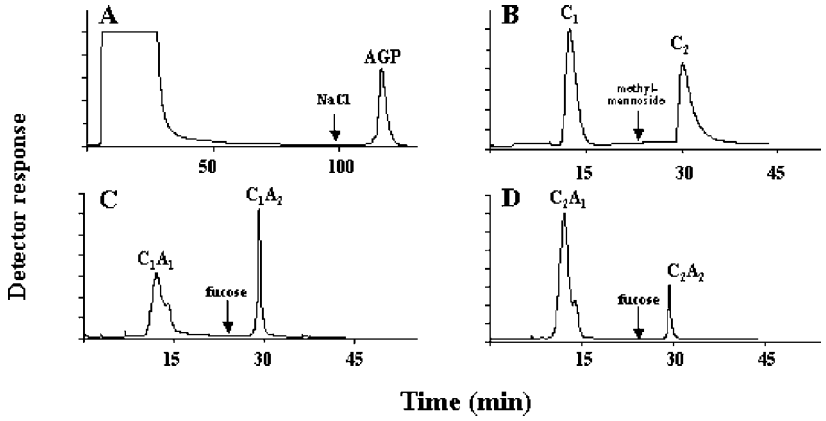

Figure 1. *Representative chromatograms of AGP isolation (A), Con A fractionation (B), AAL fractionation of the C_1 glycoform (C) and AAL fractionation of the C_2 glycoform.* See Methods for experimental details. C_1: Con A non-bound AGP fraction, C_2: Con A bound AGP fraction, A_1: AAL non-bound AGP fraction and A_2: AAL bound AGP fraction.

A each, were used to isolate Con A-non-bound (C_1) and Con A-bound (C_2) AGP-glycoforms (see Figure 1B). The non-bound C_1-fraction was collected in the void volume of the column with PBS, whereas the bound C_2-fraction could be eluted with PBS containing 33 mM α-methylmannopyranoside. The collected fractions were lyophilized and desalted as described above and dissolved in PBS. Four combined 1-ml AAL-HiTrap columns were used to separate the C_1 and C_2 fraction into AAL non-bound (C_1A_1 and C_2A_1) and AAL bound (C_1A_2 and C_2A_2) AGP-glycoforms (see Figure 1C and D). C_1A_1 and C_2A_1 were collected in the void volume of the column with PBS. C_1A_2 and C_2A_2 were eluted with PBS containing 10 mM L-fucose. The AGP-glycoforms were lyophilized, desalted and dissolved in milli-Q water. Con A and AAL that, as a result of leakage from the HiTrap columns, may have been bound to AGP was removed by subjecting the AGP samples to DEAE-Sepharose chromatography. Disposable columns with 300 μl DEAE-Sepharose were washed with 3 ml washing buffer (68.4 mM NaCl, 1.3 mM KCl, 5.1 mM Na_2HPO_4 and 0.9 mM KH_2PO_4) before binding the AGP samples on DEAE-Sepharose. After binding, the samples were washed with 6 ml washing buffer containing 33 mM α-methylmannopyranoside and 10 mM L-fucose to remove Con A and AAL. AGP was eluted by 2 ml washing buffer with an end concentration of 1.15 M NaCl followed by lyophylization and desalting as described above. Finally, the purity of each AGP-glycoform preparation was controlled by SDS-PAGE and revealed the presence of only one band. Samples of the four glycoforms were stored at -20°C until hydrolyzing and derivatization. According to the reactivity of the glycoforms with Con A and AAL [8] the five carbohydrate structures on each of the AGP-glycoforms represented: non- or lowly fucosylated tri- and tetraantennary glycans (C_1A_1), tri- and tetraantennary glycans containing a relatively high amount of fucose residues (C_1A_2), one or more diantennary glycans and maximally four non- or lowly fucosylated tri- and tetraantennary glycans (C_2A_1), and one or more diantennary glycans and maximally four tri- and tetraantennary glycans with a relatively high amount of fucose residues (C_2A_2). All glycoforms were strongly sialylated according to their electrophoretic mobility in crossed immunoelectrophoresis without a lectin in the first dimension gel (not shown).

Determination of AGP concentrations

Concentrations of total AGP and of the various AGP-glycoforms were determined by single radial immunodiffusion, according to Mancini et al. [21], using mono-specific precipitating anti-AGP antiserum. Human serum protein calibrator (HSPC) with known AGP concentration, consisting of pooled sera from healthy blood donors, was used as a standard for the determination of the AGP concentration.

Determination of [1-^{13}C]ketoisovalerate and [1-^{13}C]valine enrichments

Precursor [^{13}C]valine enrichment in the cell was estimated from its [^{13}C]keto acid enrichment in plasma. Ketoisovalerate (KIV) in plasma was analyzed as the O-*t*-butyldimethylsilylquinoxalinol derivative. The [^{13}C]KIV enrichments were measured by gas chromatography combustion isotope ratio mass spectrometry (GC-c-IRMS) on a HP Engine (Hewlett-Packard, Palo Alto, CA, USA) using positive chemical ionization [22]. Valine from the AGP hydrolysates was analyzed as the *N*-methoxycarbonyl methyl ester; [1-^{13}C]valine enrichments were measured by GC-c-IRMS [23]. Enrichment was expressed as tracer-to-tracee molar ratio (TTR).

Calculation of fractional synthesis rate

The FSRs were calculated for each individual and per glycoform according to the formula of Foster and co-workers [37]: $A(t) = \mathrm{TTR_{KIV}} * [1 - e^{-k(t-d)}]$, where $A(t)$ is the time series of the TTRs of valine in AGP-glycoform samples, $\mathrm{TTR_{KIV}}$ is the mean of the TTR of ketoisovalerate in plasma during infusion, d is a delay time, k represents the slope (FSR) of the glycoform,

and t represents the time points in the series during the 6-h [^{13}C]valine infusion. FSRs are expressed in percent per day (%.day^{-1}).

Statistical analyses

Data were analyzed with SPSS software. Student t-test and analysis of variance (ANOVA) were performed for comparing means. Regression analyses were conducted and Pearson's r was calculated to test the fits of the mono-exponential synthesis models. Values are presented as means ±SE, unless otherwise stated. A P-value <0.05 was considered significant (two-tailed).

Results

Plasma concentrations of total AGP and glycoforms

Considerable variances in relative AGP-glycoform distribution between subjects were found (Table 2). However, the means of the glycoforms taken over all subjects were significantly different compared to each other ($p < 0.0005$). From all subjects the four glycoforms were obtained, but at some time points the amount of recovered protein was too small to allow further analysis by GC-c-IRMS (cf. Table 3). Nevertheless for each glycoform at least 3–4 complete series of preparations were obtained out of the five individuals, and these were analyzed and used to calculate the FSR of the glycoforms.

Fractional synthesis rate of AGP-glycoforms

In Figure 2, the incorporation of the [^{13}C]valine tracer in AGP fractions during tracer infusions are depicted for all available time series. Tracer incorporation of [^{13}C]valine in AGP-glycoforms showed a significant fit for the monoexponential model in all persons and all fractions (proportion of explained variance: range 0.92–0.997, $0.0001 < p < 0.05$). The time delay was not statistically different from zero. The tracer-to-tracee molar ratios for ketoisovalerate (TTR_{KIV}) presented in Table 3 show that the saturation with [^{13}C]valine was within acceptable limits for all subjects.

Table 2. Relative distribution of AGP-glycoforms

		Relative distribution of AGP glycoforms (% of total AGP)			
Subject	*AGP concn. (mg/L)*	*C_1A_1*	*C_1A_2*	*C_2A_1*	*C_2A_2*
1	690 ± 80	35.5 ± 1.2	6.0 ± 1.3	54.3 ± 0.8	4.2 ± 0.9
2	990 ± 40	23.6 ± 1.0	25.7 ± 0.4	38.6 ± 0.6	12.1 ± 0.6
3	980 ± 70	19.2 ± 0.5	28.5 ± 1.1	39.1 ± 1.4	13.3 ± 1.3
4	520 ± 90	34.3 ± 1.8	14.7 ± 1.1	46.1 ± 2.7	4.9 ± 1.1
5	930 ± 60	35.9 ± 2.4	20.0 ± 1.6	37.7 ± 1.8	6.4 ± 0.8
Mean	822 ± 186	29.7 ± 7.2*	19.0 ± 8.2*	43.2 ± 6.7*	8.2 ± 3.9*

The relative glycoform distribution of the glycoforms is calculated from the recoveries after sequential Con A- and AAL-affinity chromatography (see Figure 1). The values represents the mean ± S.D. of the 6 preparations of each AGP-glycoform as obtained from the plasma at the various time points during and at the end of the [^{13}C]valine infusion. C_1A_1, not bound to Con A nor to AAL; C_1A_2, not bound to Con A but bound to AAL; C_2A_1, bound to Con A but not to AAL; C_2A_2, bound to Con A as well as to AAL.
*Percentage of glycoform significantly different as compared to the three other glycoforms (ANOVA, $P < 0.0005$).

Table 3. Fractional synthesis rates of the four AGP glycoforms and mean TTR_{KIV}

	Mean individual FSR per glycoform (%/day)				
Subject	*C1A1*	*C1A2*	*C2A1*	*C2A2*	*TTR_{KIV} (%)*
1	15.7 ± 1.2	n.d.	n.d.	13.3 ± 1.9	11.4 ± 0.8
2	n.d.	12.7 ± 1.6	15.4 ± 1.0	11.5 ± 0.8	10.2 ± 0.3
3	13.9 ± 0.3	16.4 ± 0.6	n.d.	n.d.	11.7 ± 0.6
4	15.8 ± 0.7	14.1 ± 0.8	18.0 ± 0.7	15.9 ± 4.2	8.9 ± 0.3
5	11.7 ± 0.4	13.1 ± 0.4	13.6 ± 0.5	12.1 ± 0.4	11.3 ± 0.0
Mean of Subject 1–5	14.3 ± 0.9	14.1 ± 0.7	15.7 ± 1.0	13.2 ± 0.9	

Data are expressed as mean ± S.E. of the results obtained for each of the glycoforms at the various time-points. n.d., not determined because the amount of recovered protein at some time points was too small to allow further analysis by mass spectrometry. The TTR_{KIV} values are incorporated in the table to show that the saturation with [^{13}C]valine was within acceptable limits for all subjects. See materials and methods for calculation of FSR and TTR values.

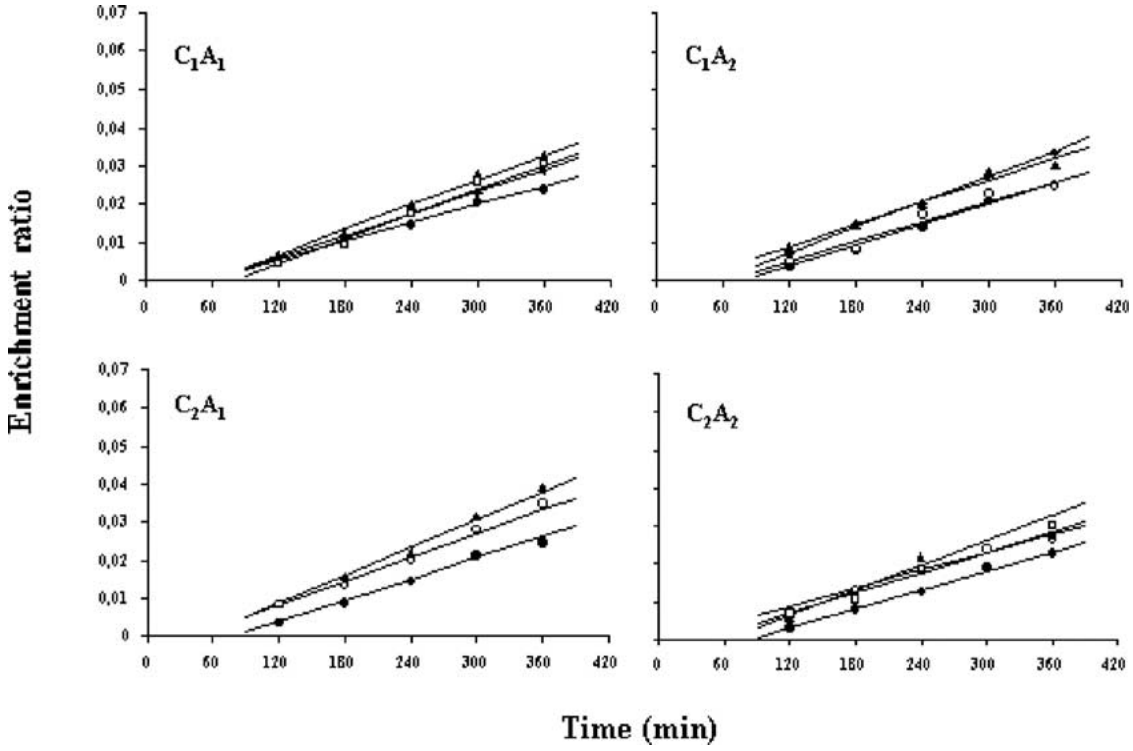

Figure 2. *Enrichment of [^{13}C]valine in AGP-glycoforms relative to precursor enrichment in human subjects.* Subject 1 (□), 2 (○), 3 (♦), 4 (▲), and 5 (●). See Methods for explanation and for calculation of FSR.

The individual FSRs of the AGP-glycoforms per subject did not differ significantly from each other (Table 3). The mean FSRs of the AGP-glycoforms of all five subjects ranged from 13.2–15.7%/day, and also lacked significant differences. The data were used to calculate the absolute synthesis rates of the various AGP-glycoforms (see legend of Figure 3) and showed a linear relationship between pool-size and absolute synthesis rate for the four AGP-glycoforms (Figure 3) with a high correlation coefficient ($R^2 = 0.9945$). The slope of this graph indicated a renewal rate of 15.9%/day for all four glycoforms.

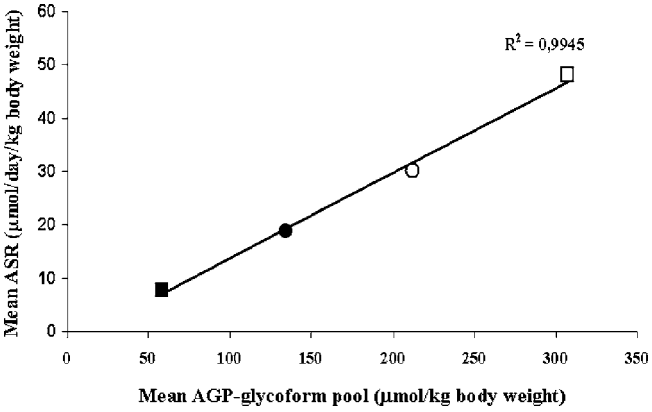

Figure 3. *Linear relationship between absolute synthesis rate (ASR) and pool size of the four AGP-glycoforms.* ASR is the product of FSR and pool size per glycoform. Pool sizes of the AGP-glycoforms were calculated from the data presented in Table 2, assuming a plasma volume of 80 ml circulating blood per kg body weight, a hematocrit of 0.45, and a molecular weight of 41,500 Dalton for AGP. The means of ASR and pool size per glycoform of the five subjects are presented: ○, C_1A_1; ●, C_1A_2; □, C_2A_1; ■, C_2A_2.

Discussion

To the best of our knowledge this is the first study describing the *in vivo* kinetics of different glycoforms for a human acute-phase glycoprotein. The highly significant linear relationship between absolute synthesis rate and plasma pool sizes of the various AGP-glycoforms (Figure 3) indicates that their relative occurrence in plasma is regulated by synthesis. This is in line with the general assumption that the post-translational glycosylation of a secretory glycoprotein is dependent on the glycosylation capacity of the synthesizing cell, i.e. on the relative activities of glycosyltransferases that are present in the Golgi system under certain conditions [24]. This can be exemplified for AGP by a study of human AGP expressed in mice. The transgenic animals appeared to be unable to fucosylate human AGP, which could be attributed to the lack of α3-fucosyltransferase activity in mice livers [19,25].

Since during the acute-phase response the hepatic mRNA expression of AGP and its synthesis and secretion is increased [1,26], it is reasonable to assume that also under those conditions the increases in specific AGP-glycoforms are dependent

on synthetic regulation. This would implicate that the glycosylation potential of the liver is subject to acute-phase regulation. Indeed, several studies have shown that inflammatory conditions can affect the activities of hepatic glycosyltransferases [6,27–32,38]. Kinetic studies of other acute-phase proteins have shown that synthetic regulation is responsible for increased plasma levels during several injuries [33–35]. One of these, fibrinogen, was studied in patients with chronic nephrosis, using the same stable isotope tracer technique as applied in the present study. The FSR for fibrinogen correlated strongly with its plasma concentration [36].

During inflammation especially the Con A- and AAL-reactive AGP-glycoforms are increased (reviewed in [8]). It has been shown that the glycan compositions of these glycoforms give rise to specific anti-inflammatory properties of AGP. Examples are inhibition of proliferation of lymphocytes [18], and amelioration of neutrophil- and complement-mediated injuries [9,17]. This implicates, that in addition to increasing the concentration of an acute-phase protein the liver would have the potency to increase the anti-inflammatory properties of that protein by changing the post-translational glycosylation process. This would fit in the generally accepted view that the hepatic acute-phase response is a feedback response to excessive inflammation and is meant to dampen down side effects [2,26]. In addition to the findings of the present study, studying the synthetic kinetics of the AGP glycoforms in patients afflicted by a chronic inflammatory status could improve our understanding of the pathophysiologic role of AGP.

References

1 Hochepied T, Berger FG, Baumann H, Libert C, α_1-Acid glycoprotein: an acute phase protein with inflammatory and immunomodulating properties, *Cytokine Growth Factor Rev* **14**, 25–34 (2003).

2 Kushner I, The phenomenon of the acute-phase response, *Ann NY Acad Sci* **389**, 39–48 (1982).

3 Schmid K, Nimberg RB, Kimura A, Yamaguchi H, Binette JP, The carbohydrate units of alpha1-acid glycoprotein, *Biochim Biophys Acta* **492**, 291–302 (1977).

4 Treuheit MJ, Costello CE, Halsall HB, Analysis of the five glycosylation sites of human AGP, *Biochem J* **283**, 105–12 (1992).

5 Wieruszeski JM, Fournet B, Konan D, Durand G, 400-MHz 1H-NMR spectroscopy of fucosylated tetrasialyloligosaccharides from normal and cirrhotic AGP, *FEBS Lett* **238**, 390–4 (1988).

6 Brinkman-Van der Linden ECM, Mollicones R, Oriol R, Larson G, Van den Eijnden DH, Van Dijk W, A Missence mutation in the FUT6 gene results in total absence of α3-fucosylation of human alpha1-acid glycoprotein, *J Biol Chem* **271**, 14492–5 (1996).

7 De Graaf T, Van der Stelt ME, Anbergen GM, Van Dijk W, Inflammation-induced expression of sialyl Lewis-x-containing glycan structures on α_1-acid glycoprotein (orosomucoid) in human sera, *J Exp Med* **177**, 657–66 (1993).

8 Van Dijk W, Brinkman-Van der Linden ECM, Havenaar EC, Glycosylation of α_1-acid glycoprotein (orosomucoid) in health and disease: occurrence, regulation and possible functional implications, *Trends Glycosci Glycotechnol* **53**, 235–45 (1998).

9 Van Dijk W, α_1-Acid glycoprotein: a natural occurring anti-inflammatory molecule? *Adv Exp Med Biol* **376**, 223–9 (1995).

10 Brinkman-Van der Linden ECM, Van Ommen ECR, Van Dijk W, Glycosylation of α_1-acid glycoprotein in septic shock: changes in degree of branching and in expression of sialyl Lewis-x groups, *Glycoconjugate J* **13**, 27–31 (1996).

11 Havenaar EC, Dolhain RJEM, Turner GA, Goodarzi MT, Van Ommen EC, Breedveld FC, Van Dijk W, Do synovial fluid acute phase proteins from patients with rheumatoid arthritis originate from serum, *Glycoconjugate J* **14**, 457–65 (1997).

12 Pos O, Van der Stelt ME, Wolbink GJ, Nijsten MWN, Van der Tempel GL, Van Dijk W, Changes in the serum concentration and glycosylation of α_1-acid glycoprotein and alpha1-protease inhibitor in severely burned patients: relation to interleukin-6 levels, *Clin Exp Immunol* **82**, 579–82 (1990).

13 Hervé F, Duche JC, Dathis P, Marche C, Barre J, Tillement JP, Binding of diisopyramide, methadone, dipyridamole, chlorpromazine, lidocaine and progesterone to the two main genetic variants of human alpha1-acid glycoprotein. Evidence for drug-binding difference between the variants and for the presence of two separate drug-binding sites on the α_1-acid glycoprotein, *Pharmacogenetics* **6**, 403–15 (1996).

14 Pervaiz S, Brew K, Homology and structure-function correlations between α_1-acid glycoprotein and serum retinol-binding protein and its relatives, *FASEB J* **1**, 209–14 (1987).

15 Costello M, Fiedel BA, Gewurz H, Inhibition of platelet aggregation by native and desialylated alpha1-acid glycoprotein, *Nature* **281**, 677–8 (1979).

16 Pos O, Moshage HJ, Yap SH, Snieders JPMALA, Van Gool J, Boers W, Brugman AM, Van Dijk W, Effects of monocytic products, recombinant interleukin-1 and recombinant interleukin-6 on the glycosylation of α_1-acid glycoprotein: studies with primary human hepatocyte cultures and rats, *Inflammation* **13**, 415–24 (1989).

17 Williams JP, Weisser MR, Pechet TTV, Kobzik L, Moore FD, Hechtman HB, α_1-Acid glycoprotein reduces local and remote injuries after intestinal ischemia in the rat, *Am J Physiol* **273**, G1031–5 (1997).

18 Pos O, Oostendorp RAJ, Van der Stelt ME, Scheper RJ, Van Dijk W, Con A-non reactive human α_1-acid glycoprotein (AGP) is more effective in modulation of lymphocyte proliferation than Con A-reactive AGP serum variants, *Inflammation* **14**, 133–41 (1990).

19 Havenaar EC, Hoff RC, Van den Eijnden DH, Van Dijk W, Sialyl Lewis^X epitopes do not occur on acute phase proteins in mice: relationship to the absence of α3-fucosyltransferase in the liver, *Glycoconjugate J* **15**, 389–95 (1998).

20 McGuire EAM, Helderman JH, Tobin JD, Andres R, Berman M, Effects of arterial versus venous sampling on analysis of glucose kinetics in man, *J Appl Physiol* **41**, 565–7 (1976).

21 Mancini G, Carbonara AO, Heremans JF, A single radial diffusion method for the immunological quantification of proteins, *Immunochemistry* **2**, 235–54 (1965).

22 Kulik W, Jacobs C, De Meer K, Determination of extracellular and intracellular enrichments of α-[1-13C]ketoisovalerate using enzymatically converted [1-13C]valine standardization curves and gas chromatography-mass spectrometry, *J Chromatogr B* **729**, 211–6 (1999).

23 Kulik W, Meesterburrie JAN, Jakobs C, De Meer K, Determination of delta-^{13}C values of valine in protein hydrolysate by gas

chromatography-combustion isotope ratio mass spectrometry, *J Chromatogr B* **710**, 37–47 (1998).
24 Schachter H, *The joys of HexNAc*. The synthesis and function of N- and O-glycan branches, *Glycoconjugate J* **17**, 465–83 (2000).
25 Van Dijk W, Pos O, Van der Stelt ME, Moshage HJ, Yap SH, Dente L, Baumann P, Eap CB, Inflammation-induced changes in expression and glycosylation of genetic variants of α_1-acid glycoprotein (AGP): studies with human sera, primary cultures of human hepatocytes and transgenic mice, *Biochem J* **276**, 343–7 (1991).
26 Baumann H, Gauldie J, The acute phase response, *Immunol Today* **15**, 74–80 (1994).
27 Dalziel M, Lemaire S, Ewing J, Kobayashi L, Lau JT, Hepatic acute phase induction of murine β-galactoside α2,6-sialyltransferase (ST6Gal I) is IL-6 dependent and mediated by elevation of exon H-containing class of transcripts, *Glycobiology* **9**, 1003–8 (1999).
28 Jamieson JC, McCaffrey G, Harder PG, Sialyltransferase: a novel acute-phase reactant, *Comp Biochem Physiol B* **105**, 29–33 (1993).
29 Lombart C, Sturgess J, Schachter H, The effect of turpentine-induced inflammation on rat liver glycosyltransferases and Golgi complex ultrastructure, *Biochim Biophys Acta* **629**, 1–12 (1980).
30 Memon RA, Holleran WM, Uchida Y, Moser AH, Ichikawa S, Hirabayashi Y, Grunfeld C, Feingold KR, Regulation of glycosphingolipid metabolism in liver during the acute phase response, *J Biol Chem* **274**, 19707–13 (1999).
31 Tanaka S, Yazawa S, Noguchi K, Nishimura T, Miyanaga K, Kochibe N, Poland DCW, Van Dijk W, Matta KL, Molecular analysis of plasma α1, 3-fucosyltransferase deficiency and development of the methods of genotyping, *Exptl Clin Immunogen* **18**, 1–12 (2001).
32 Van Dijk W, Boers W, Sala M, Lasthuis AM, Mookerjea S, Activity and secretion of sialyltransferase in primary monolayer cultures of rat hepatocytes cultured with and without dexamethasone, *Biochem Cell Biol* **64**, 79–84 (1986).
33 Jahoor F, Gazzard B, Philips G, Sharpstone D, Delrosario M, Frazer ME, Heard W, Smith R, Jackson A, The acute phase response to human immunodeficiency virus infection in human subjects, *Am J Physiol* **276**, E1092–8 (1999).
34 Mansoor O, Cayol M, Gachon P, Boirie Y, Schoeffler P, Obled C, Beaufrere B, Albumin and fibrinogen synthesis increase while muscle protein synthesis decreases in head-injured patients, *Am J Physiol* **273**, E898–E902 (1997).
35 Preston T, Slater C, McMillan DC, Falconer JS, Shenkin A, Fearon KC, Fibrinogen synthesis is elevated in fasting cancer patients with an acute phase response, *J Nutr* **128**, 1355–60 (1998).
36 De Sain-van der Velden MGM, Kaysen GA, De Meer K, Stellaard F, Voorbij AM, Reijngoud DJ, Rabelink TJ, Koomans HA, Proportionate increase of fibrinogen and albumin synthesis in nephrotic patients: measurements with stable isotopes, *Kidney Int* **53**, 181–8 (1998).
37 Foster DM, Barrett PH, Toffolo G, Beltz WF, Cobelli C, Estimating the fractional synthetic rate of plasma apolipoproteins and lipids from stable isotope data, *J Lipid Res* **34**, 2193–205 (1993).
38 Woloski BM, Gospodarek E, Jamieson JC, Studies of monokines as mediators of the acute phase response: effects on sialyltransferase, albumin, α_1-acid glycoprotein and β-N-acetylhexosaminidase, *Biochem Biophys Res Commun* **130**, 30–6 (1985).

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