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Serum levels of immunoglobulins (IgG, IgA, IgM) in a general adult population and their relationship with alcohol consumption, smoking and common metabolic abnormalities

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Summary

The present study investigated serum immunoglobulin (Ig) concentrations in relation to demographic factors, common habits (alcohol consumption and smoking) and metabolic abnormalities in an adult population-based survey including 460 individuals. Serum levels of interleukin (IL)-6, a marker of inflammation, were also determined. After adjusting for confounders, male sex was associated positively with IgA levels and negatively with IgM levels. Age was associated positively with IgA and IgG levels. Smoking was associated negatively with IgG levels. Heavy drinking was associated positively with IgA levels. Metabolic abnormalities (obesity and metabolic syndrome) were associated positively with IgA levels. Abdominal obesity and hypertriglyceridaemia were the components of metabolic syndrome associated most strongly with serum IgA. Heavy drinkers with metabolic syndrome showed particularly high serum IgA levels. Serum IL-6 levels were correlated positively with IgA and IgG concentrations. It is concluded that sex, age, alcohol consumption, smoking and common metabolic abnormalities should be taken into account when interpreting serum levels of IgA, IgG and IgM.

Keywords: alcohol, immunoglobulins, metabolic syndrome, obesity, smoking

Introduction

Serum immunoglobulin levels are determined routinely in clinical practice because they provide key information on the humoral immune status. Low immunoglobulin (Ig) levels define some humoral immunodeficiencies [1]. In contrast, high immunoglobulin levels (polyclonal gammopathy) are observed in liver diseases, chronic inflammatory diseases, haematological disorders, infections and malignancies [2]. Moreover, immunoglobulin levels aid in the diagnosis of some disorders, particularly liver diseases [3–7].

Determining the distribution of immunoglobulin levels in general populations is important for interpreting reference values. The common guidelines for definition and determination of reference intervals in the clinical laboratory note that partitioning should be considered when there are significant differences among subgroups defined by age, sex and common exposures such as smoking or alcohol consumption [8–10]. However, studies focusing upon the possible influences of these factors on serum immunoglobulin levels are scarce. It has been reported that IgM levels are higher in females than in males [11–13]. Also, serum immunoglobulin concentrations tend to increase with age [11,14]. It is well known that heavy drinkers with advanced liver disease often present with high IgA values [5,15–17], but fewer studies have addressed the effects of smoking and alcohol intake (from light to heavy) *per se* on serum IgA, IgG or IgM [18].

An increase in serum IgA levels is a generalized phenomenon in diabetic patients [19,20]. Chronic inflammation is a key feature of type 2 diabetes, obesity and metabolic syndrome [21,22], a cluster of abnormalities characterized by insulin resistance along with specific risk factors including hyperglycaemia, visceral adiposity, dyslipidaemia and high blood pressure [23,24]. Production of proinflammatory cytokines is increased in patients with metabolic syndrome [25,26]. These include adipocytokines such as interleukin (IL)-6 [25,27], which is a co-factor for immunoglobulin synthesis [28–30] and a common marker of inflammation [31]. Obesity and metabolic syndrome, the paradigms of metabolic abnormalities, are common in many populations, and their worldwide prevalences have increased dramatically during recent decades. To the best of our knowledge, no previous study has been focused upon a possible association of these common metabolic abnormalities with serum immunoglobulin levels.

Taken together, these data emphasize the need for multivariate analyses in order to detect confounding or

interactions among all these factors that are associated with each other as well as with immunoglobulin levels. In this adult population-based study, we investigated serum immunoglobulin (IgA, IgG and IgM) levels in relation to (i) demographic factors (age and sex); (ii) common environmental exposures (alcohol consumption and smoking); and (iii) common metabolic abnormalities, including the components of metabolic syndrome. In addition, we investigated the possible relationship between immunoglobulin concentrations and serum levels of IL-6.

Methods

Study population

The present study took advantage of a survey of the general adult population from the municipality of A-Estrada, in north-western Spain. The study was intended primarily to investigate immunological alterations associated with alcohol consumption. Detailed descriptions of study methodology and population sample characteristics have been reported elsewhere [32]. Briefly, an age-stratified random sample ($n = 720$) of the adult population (> 18 years of age) of the municipality was drawn from the Health Care Registry, which covers > 95% of the population. A total of 469 individuals consented to participate. Of these, a serum sample for immunoglobulin determination (see below) was available for 460 individuals. The median age of these individuals was 54 years (range 18–92 years). All participants were Caucasians. A total of 203 (44·1%) were males.

Classification of alcohol consumption and smoking

Alcohol consumption was evaluated by the system of standard drinking units [33], which sums the number of glasses of wine (~ 10 g), bottles of beer (~ 10 g) and units of spirits (~ 10 g) consumed regularly per week. Individuals with habitual alcohol consumption of 1–140 g/week ($n = 140$, 30·4%) were considered light drinkers, those with alcohol consumption of 141–280 g/week ($n = 53$, 11·5%) were considered moderate drinkers and those with alcohol consumption of > 280 g/week ($n = 46$, 10·0%) were considered heavy drinkers. The remainder ($n = 221$, 48·0%), alcohol abstainers or very occasional alcohol drinkers, were included in the same group. Consumers of at least one cigarette per day were considered smokers. Individuals who had quit smoking during the preceding year were still considered smokers.

Definition of metabolic abnormalities

The body mass index (BMI) was calculated as the weight (in kg) divided by the square of the height (in metres). Following standard criteria, individuals were classified as normal weight (< 25 kg/m^2), overweight (25–30 kg/m^2) or obese (> 30 kg/m^2).

Metabolic syndrome was defined by the Adult Treatment Panel III criteria [23] that include: (i) abdominal obesity (waist circumference > 102 cm in males or > 88 cm in females); (ii) hypertriglyceridaemia (fasting serum triglycerides ≥ 150 mg/dl); (iii) low high-density lipoprotein (HDL)-cholesterol levels (fasting HDL-cholesterol < 40 mg/dl in males or < 50 mg/dl in females); (iv) increased blood pressure (arterial blood pressure ≥ 130/≥ 85 mmHg or current anti-hypertensive medication use); and (v) hyperglycaemia (fasting serum glucose ≥ 110 mg/dl or current anti-diabetic therapy). Individuals who met at least three of these criteria ($n = 114$, 24·8%) were classified as having metabolic syndrome, as reported previously [34]. Missing data included BMI in one case, waist circumference in two cases, serum triglycerides in one case and HDL-cholesterol in one case. The remaining criteria were enough to classify these cases as having or lacking metabolic syndrome. Routine laboratory determinations were performed with an Olympus AU-400 analyser (Olympus, Tokyo, Japan).

Specific laboratory determinations

Serum immunoglobulins (IgG, IgA and IgM) levels were determined by a commercial nephelometry assay using a BN-II device (Dade Behring, Marburg, Germany). The manufacturer indicates the following reference intervals for healthy adults: IgA 70–400 mg/dl, IgG 700–1600 mg/dl and IgM 40–230 mg/dl [35].

Serum IL-6 was determined by a commercial chemiluminescent enzyme immunoassay (Immulite^{TM}; DPC, LA, CA, USA). The lower threshold for detection of IL-6 with this method is 2 pg/ml. For the present study, serum levels > 10 pg/ml (corresponding to the ~95th percentile in the studied population) were considered abnormally high.

Statistical analyses

Cross-tabulation significance levels were based on second-order corrected Pearson's χ^2 for categorical variables. The Mann–Whitney *U*-test, the Kruskal–Wallis test and the Jonckheere–Terpstra test (for trend analysis) were employed for comparison of quantitative variables. In some descriptions, the 2·5th and the 97·5th percentiles were given as measures of dispersion as an approach to reference values [9]. Serum IL-6 concentrations were considered in categories because the majority of individuals presented with undetectable IL-6 levels and the distribution of values was highly skewed to the right. Linear regression was employed for multivariate analyses with serum immunoglobulin levels as dependent variables. For covariates, age (in years) entered the equation as a quantitative variable, and binary variables entered the equation as '1' ('present' or 'yes') or '0' ('absent' or 'no'). Dummy variables were created for non-binary categorical variables, using the lowest category as the reference. Variables were forced to enter the equation in all models. To

account for the stratified sampling, a design-based analysis including compensatory weights was performed for the estimation of immunoglobulin levels in the overall population. The Stata 7·0 package (Stata Corp., College Station, TX, USA) and the SPSS package (SPSS Inc., Chicago, IL, USA) were employed. Two-tailed *P*-values < 0·05 were considered statistically significant.

Ethical considerations

All subjects consented to participate in the study. The study conformed to the Helsinki Declaration and was reviewed and approved by the local Research Committee.

Results

Overall distribution of serum immunoglobulin levels in the population

The levels of all three immunoglobulins (IgA, IgG, IgM) approached a normal distribution, although slightly skewed to the right (Fig. 1). The only case of frank immunoglobulin deficiency was a female with nearly undetectable IgA levels (weighted prevalence, 0·2%, 95% CI 0–0·6%).

Relation of serum immunoglobulin levels with age and sex

Serum IgA levels were higher in males than in females (Table 1). Conversely, serum IgG and serum IgM levels were higher in females than in males (Table 1). These differences between sexes were observed throughout all age ranges (Fig. 2). Serum IgA and serum IgG levels tended to increase with age (*P* for trend < 0·001 in both cases). This trend was observed in both sexes (Fig. 2). Serum IgM showed no significant variation with age (*P* for trend = 0·54) (Fig. 2).

Relation of serum immunoglobulin levels with alcohol consumption and smoking

Serum IgA levels tended to increase in parallel with alcohol consumption (*P* for trend < 0·001) (Table 1). Accordingly, the highest IgA levels were observed in heavy drinkers, who exhibited significantly higher IgA levels than those of abstainers ($P < 0·001$), light drinkers ($P = 0·001$) and moderate drinkers ($P = 0·04$). In contrast, IgG and IgM levels did not increase with alcohol consumption. Indeed, IgG levels tended to be lower in moderate consumers than in abstainers (Table 1).

Serum IgG levels were lower in smokers than in non-smokers ($P < 0·001$) (Table 1). Serum levels of IgA and IgM in smokers were not significantly different from those of non-smokers (Table 1).

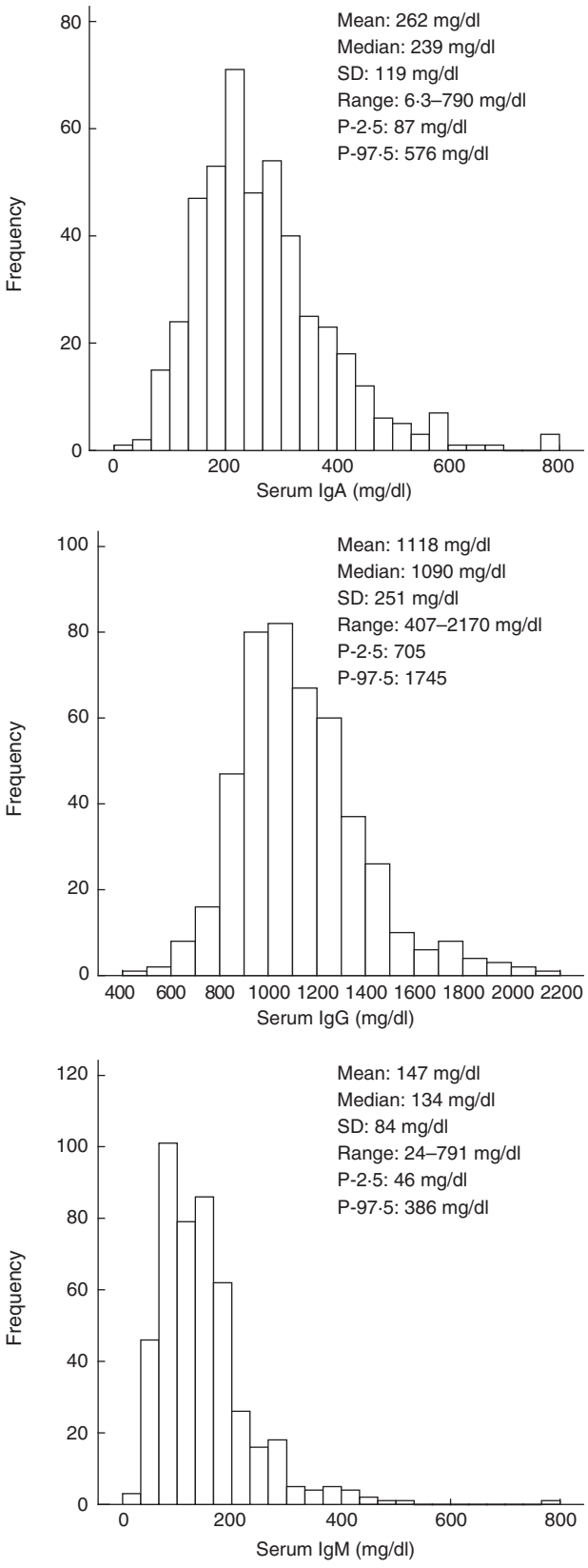

Fig. 1. Histograms of serum immunoglobulin levels in the population studied. The calculation of means, medians, standard deviations (s.d.), 2·5th and 97·5th percentiles ($P = 2·5$ and $P = 97·5$, respectively) were weighted according to the study design.

Table 1. Serum immunoglobulin (Ig) levels in relation to sex, alcohol consumption, and smoking.

	No.	IgA (mg/dl)	IgG (mg/dl)	IgM (mg/dl)
Sex				
Female (reference)	257	228 (82–470)	1120 (694–1760)	147 (50–398)
Male	203	274 (89–624)***	1060 (701–1803)*	112 (40–305)***
Alcohol consumption†				
Abstainers (reference)	221	231 (92–503)	1120 (680–1818)	137 (44–375)
Light drinkers	140	239 (78–579)	1095 (742–1784)	131 (48–386)
Moderate drinkers	53	263 (54–733)	1050 (599–1625)*	130 (36–416)
Heavy drinkers	46	318 (63–569)***	1075 (466–1922)	124 (40–509)
Smoking				
Non-smokers (reference)	361	250 (89–564)	1110 (741–1760)	132 (46–367)
Smokers	99	224 (74–730)	995 (628–1775)***	137 (43–428)

Data are medians and 2·5th–97·5th percentile ranges (within parentheses). *$P < 0·05$, ***$P < 0·001$ with respect to the reference category. †Individuals with alcohol consumption of 1–140 g/week were considered light drinkers, those with alcohol consumption of 141–280 g/week were considered moderate drinkers and those with alcohol consumption > 280 g/week were considered heavy drinkers. Alcohol abstainers and very occasional alcohol drinkers were included in the same category.

Relation of serum immunoglobulin levels with common metabolic abnormalities

Obese individuals showed higher serum IgA levels than individuals with normal weight ($P = 0·006$) or overweight ($P = 0·005$) (Table 2). In univariate analyses, increased serum IgA levels were found in individuals with almost any component of metabolic syndrome, i.e. abdominal obesity, hypertriglyceridaemia, hyperglycaemia or high blood pressure (Table 2). Therefore, serum IgA levels were significantly higher in individuals with metabolic syndrome than among patients without it (Table 2). IgG and IgM levels showed no consistent relationship with metabolic abnormalities, although individuals with dyslipidaemia (either hypertriglyceridaemia or low HDL-cholesterol levels) exhibited higher IgM levels than individuals without it. Individuals with high blood pressure also showed higher IgG, but this association became less strong after adjusting for age and sex (see below).

Multivariate analyses of factors associated with serum immunoglobulin levels

A multiple linear regression model that adjusted for age, sex, alcohol intake, smoking status and metabolic syndrome confirmed that serum IgA levels were associated positively and independently with male sex ($P < 0·001$), ageing ($P = 0·001$), heavy drinking ($P = 0·02$) and metabolic syndrome ($P < 0·001$). Furthermore, the presence of metabolic syndrome tended to modify the effect of heavy drinking on serum IgA levels and vice versa, with IgA levels being particularly high among individuals with both conditions (Fig. 3). When the five components of metabolic syndrome were introduced to the model, hypertriglyceridaemia and abdominal obesity were the only components of the syndrome that maintained significant associations with IgA levels ($P = 0·001$ and $P = 0·02$, respectively). In the same model, hyperglycaemia showed borderline significance ($P = 0·10$). These three metabolic factors (abdominal obesity, hypertriglyceridaemia and hyperglycaemia) had an additive effect on serum IgA levels (Fig. 4).

In a similar linear regression model, serum IgG levels were associated positively and independently with ageing ($P = 0·001$). In addition, IgG levels were associated negatively and independently with moderate drinking ($P = 0·01$) and with smoking ($P = 0·02$). Serum IgM levels were associated positively and independently with female sex ($P < 0·001$).

Relation of serum IL-6 levels with serum immunoglobulin concentrations

A total of 355 individuals (77·2%) had undetectable (< 2 pg/ml) serum IL-6 levels, 77 (16·7%) had IL-6 levels of 2–10 pg/ml and the remaining 28 (6·1%) had high (> 10 pg/ml) IL-6 levels. Serum IgA and IgG (but not IgM) tended to increase in parallel with these categories of serum IL-6 levels (P for trend = 0·01, 0·03 and 0·59, respectively) (Fig. 5). No clear-cut associations were observed between IL-6 levels and sex, alcohol consumption, smoking or any of the common metabolic abnormalities studied (data not shown). The proportion of individuals with detectable ($\geq$ 2 pg/ml) IL-6 levels tended to increase with age, from 11·1% in individuals aged 18–30 years to 31·9% in those older than 80 years (P for trend = 0·03). The association between age (in years) and detectable IL-6 levels was still present after adjusting for sex, alcohol intake, smoking and metabolic syndrome in a logistic regression model [odds ratio (OR) 1·014, 95% confidence interval (CI) 1·001–1·028, $P = 0·03$].

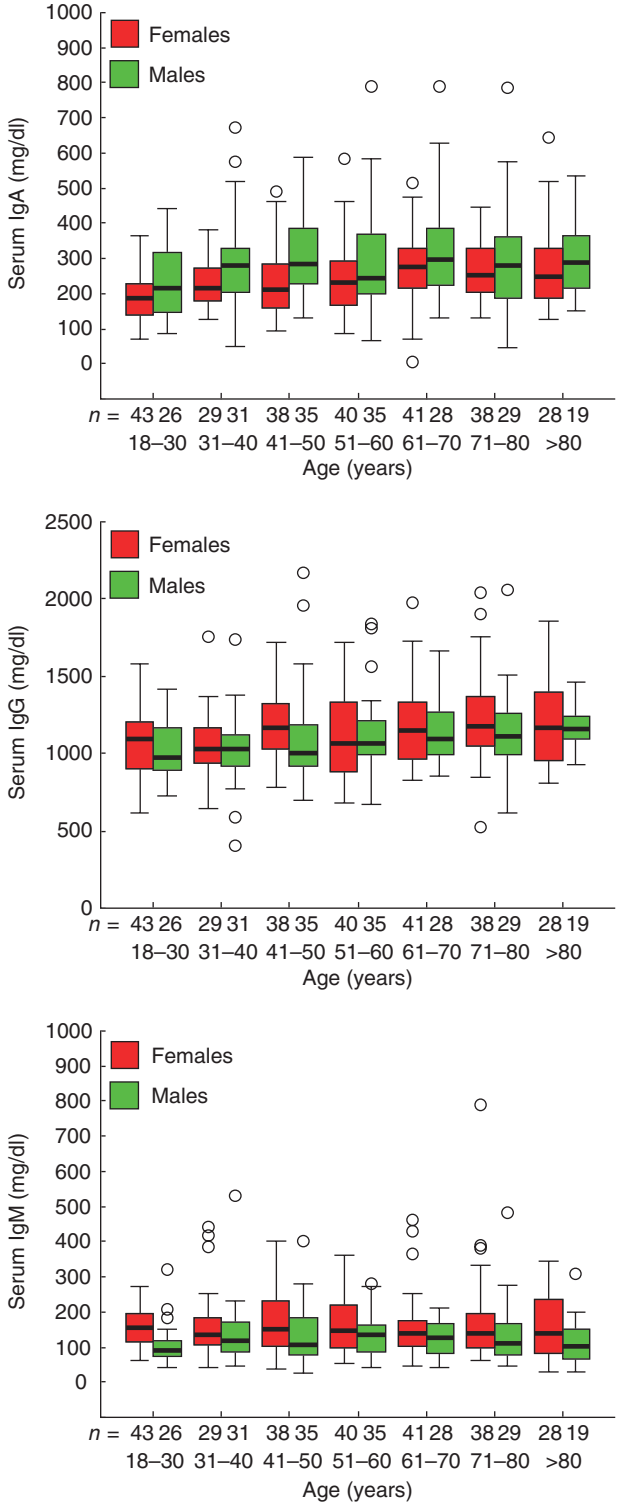

Fig. 2. Serum immunoglobulin levels in relation to sex and age strata. Serum immunoglobulin A (IgA) levels were higher in males than in females in all age strata ($P < 0{\cdot}001$), and also tended to increase with age (P for trend $< 0{\cdot}001$). Serum IgG levels were higher in females than in males in all age strata ($P < 0{\cdot}05$), and tended to increase with age (P for trend $< 0{\cdot}001$). Serum IgM were higher in females than in males in all age strata ($P < 0{\cdot}001$), but showed no significant variation with age.

Discussion

This comprehensive study in a general adult population shows that serum concentrations of serum immunoglobulins vary widely with age, sex, common habits and metabolic abnormalities. According to our results, median IgA values may be 20% higher in males, whereas median IgM values may be 30% higher in females. Sex differences in immunoglobulin concentrations, specifically high IgM levels in females, have been attributed to hormonal effects on B lymphocytes [36,37]. Such sex differences may be particularly important when interpreting immunoglobulin levels in diseases with unequal sex distribution. For instance, increased serum IgM levels are a characteristic diagnostic feature of primary biliary cirrhosis, which has a strong female predominance [4,6,7]. Also, significant increases in serum IgA and IgG were observed with age. Such increases may reflect the accumulation of chronic inflammatory conditions with ageing. In fact, there was some parallelism between IgA and IgG concentrations and serum concentrations of IL-6, a marker of inflammation and a co-factor for immunoglobulin synthesis [28–30]. In addition, the present results show that sex- and age-related changes in immunoglobulin concentrations are independent of potential confounders such as smoking, alcohol consumption and common metabolic abnormalities.

The observed variations in serum immunoglobulins with alcohol consumption and smoking confirm those reported by McMillan *et al.* in the Irish population [18]. In addition, the present study shows that these findings are independent of potential confounders. Serum IgA levels tended to increase with alcohol consumption, being higher in heavy drinkers than in light-to-moderate drinkers and abstainers. It is known that serum IgA levels are higher in alcoholic cirrhosis than in non-cirrhotic alcoholic liver disease [5,15–17], suggesting that alcoholic liver disease *per se* plays a role in IgA elevation. In fact, alcoholic liver disease has been defined as an IgA-associated disorder [38]. However, as shown in the present and previous studies [18,39], a milder IgA increase is found in heavy drinking individuals with minimal or no liver disease, suggesting that chronic alcohol intake *per se* also contributes to increased serum IgA values. Of note, IgA increase in heavy drinkers is selective, not affecting IgM or IgG. Moreover, serum IgG concentrations tend to be lower in moderate alcohol consumers than in abstainers. This finding is consistent with previous reports [18] and is independent from smoking, which is also associated with low IgG levels, as shown here and in previous studies [18]. Taken together, these findings confirm that both smoking [40] and alcohol consumption [41] have significant immunomodulatory effects.

There have been previous reports of increased serum IgA concentrations in diabetic patients [19,20], but to our knowledge there were no previous studies examining IgA concentrations in patients with metabolic syndrome, an

Table 2. Serum immunoglobulin levels in relation to common metabolic abnormalities.

	No.	IgA (mg/dl)	IgG (mg/dl)	IgM (mg/dl)
Body mass index†				
Normal weight (reference)	125	233 (80–531)	1050 (713–1852)	141 (42–412)
Overweight	194	238 (90–588)	1095 (734–1760)	126 (46–370)
Obese	140	270 (87–583)**	1130 (676–1777)*	129 (40–394)
Abdominal obesity‡				
No (reference)	328	232 (88–519)	1085 (726–1755)	136 (46–389)
Yes	130	270 (98–585)***	1130 (674–1789)	127 (39–397)
Hyperglycaemia‡				
No (reference)	349	233 (87–523)	1090 (718–1760)	136 (46–395)
Yes	111	278 (84–696)***	1080 (677–1928)	123 (33–370)
Hypertriglyceridaemia‡				
No (reference)	375	233 (88–527)	1090 (702–1760)	128 (42–375)
Yes	84	293 (76–668)***	1065 (686–1880)	148 (65–435)**
Low HDL-cholesterol levels‡				
No (reference)	330	238 (81–573)	1090 (689–1782)	122 (41–377)
Yes	129	254 (106–583)	1130 (704–1760)	149 (62–430)***
High blood pressure‡				
No (reference)	144	214 (84–427)	1040 (698–1502)	141 (43–396)
Yes	316	264 (89–583)***	1110 (697–1841)**	129 (45–385)
Metabolic syndrome‡				
No (reference)	346	228 (84–522)	1090 (735–1773)	133 (42–384)
Yes	114	298 (139–635)***	1120 (667–1778)	137 (60–408)

Data are medians and 2·5th percentile–97·5th percentile ranges (within parentheses). *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$ with respect to the reference category. †Normal weight, body mass index (BMI) < 25 kg/m^2; overweight, BMI 25–30 kg/m^2; obese, BMI > 30 kg/m^2. ‡Criteria from the Adult Treatment Panel III [23] for the definition of metabolic syndrome: abdominal obesity, defined by waist circumference > 102 cm in males or > 88 cm in females; hypertriglyceridaemia, defined by fasting serum triglycerides ≥ 150 mg/dl; low high-density lipoprotein (HDL)-cholesterol levels, defined by fasting HDL-cholesterol < 40 mg/dl in males or < 50 mg/dl in females; high blood pressure, defined by blood pressure ≥ 130/≥ 85 mmHg or current anti-hypertensive medication use; and hyperglycaemia, defined by fasting blood glucose ≥ 110 mg/dl or current anti-diabetic therapy. Individuals meeting at least three of these criteria were considered to have metabolic syndrome.

insulin-resistant state characterized by a constellation of metabolic abnormalities [23,24]. The nominal definition of metabolic syndrome is constantly changing [42], and for this reason the components of the metabolic syndrome were considered separately. Increased IgA concentrations tended to be associated with hyperglycaemia, but associations with additional components of metabolic syndrome such as hypertriglyceridemia and abdominal obesity were even stronger. These three factors had an additive effect on serum IgA levels. Immunoglobulin elevation was selective, affecting only the IgA class, with the exception of an IgM increase in individuals with dyslipidaemia. The mechanisms of IgA elevation in individuals with obesity and metabolic syndrome are unknown, but IgA elevation is not surprising because both conditions are chronic inflammatory disorders [21,22]. Serum levels of IL-6, an inflammatory marker, were associated with those of serum IgA. However, serum IL-6 levels were not found to be associated with metabolic abnormalities in the present series. Elevated IgA levels in patients with obesity and metabolic syndrome could be of clinical importance in relation to IgA-related disorders. It is well known that obesity and metabolic syndrome may accompany and worsen IgA nephropathy [43–45]. The possible role of increased IgA concentrations in the development or progression of IgA nephropathy in patients with these metabolic disorders should be investigated further. Interestingly, we observed an epidemiological interaction between heavy drinking and metabolic syndrome in relation to IgA levels, the latter being particularly high in individuals with both conditions. Of note, some parallels exist between obesity/metabolic syndrome and the consequences of heavy drinking in that both are chronic inflammatory disorders, share nearly identical histological liver findings (steatohepatitis) [46], are associated with IgA nephopathy [43–45,47] and are associated with increased serum IgA levels, as reported here. Furthermore, metabolic abnormalities modify the effect of heavy drinking in the development of alcoholic liver disease, the latter being more frequent and severe among patients with obesity/metabolic syndrome [48]. Further studies are also needed in order to ascertain the mechanisms of biological interactions between alcohol consumption and obesity/metabolic syndrome as IgA-related conditions.

In summary, the present study shows that serum concentrations of the main immunoglobulin isotypes may be affected by common factors. Serum IgA is associated positively with age, male sex, heavy drinking, obesity and

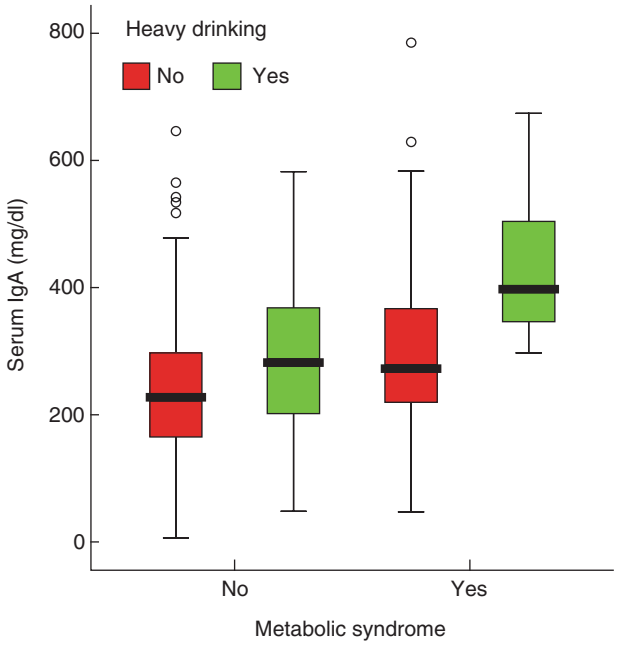

Fig. 3. Serum immunoglobulin A (IgA) levels in relation to the presence of metabolic syndrome and heavy drinking. Serum IgA levels were increased in heavy drinkers and in patients with metabolic syndrome, but were particularly high in heavy drinkers with metabolic syndrome ($P < 0{\cdot}001$ with respect to every other category).

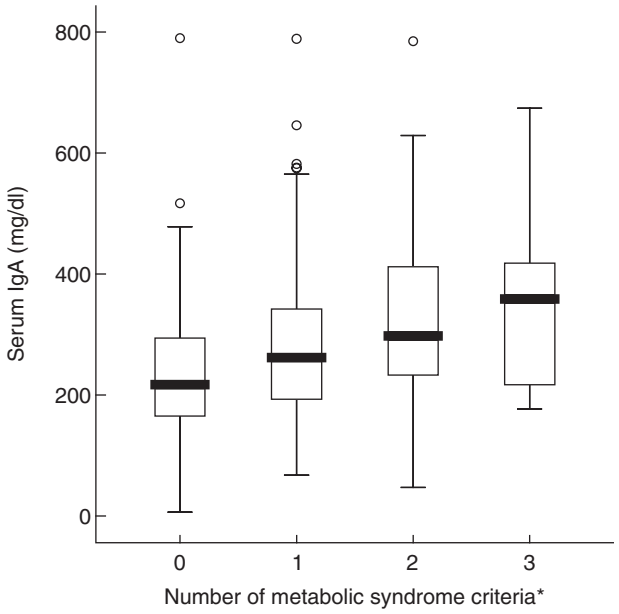

Fig. 4. Serum immunoglobulin A (IgA) levels in relation to the number of metabolic syndrome criteria [23]. Serum IgA levels tended to increase as the number of criteria increased (P for trend $< 0{\cdot}001$). *Criteria considered here included only abdominal obesity, hypertriglyceridaemia and hyperglycaemia, three of the five criteria of metabolic syndrome [23] that showed an independent association with serum IgA levels or a trend toward and association.

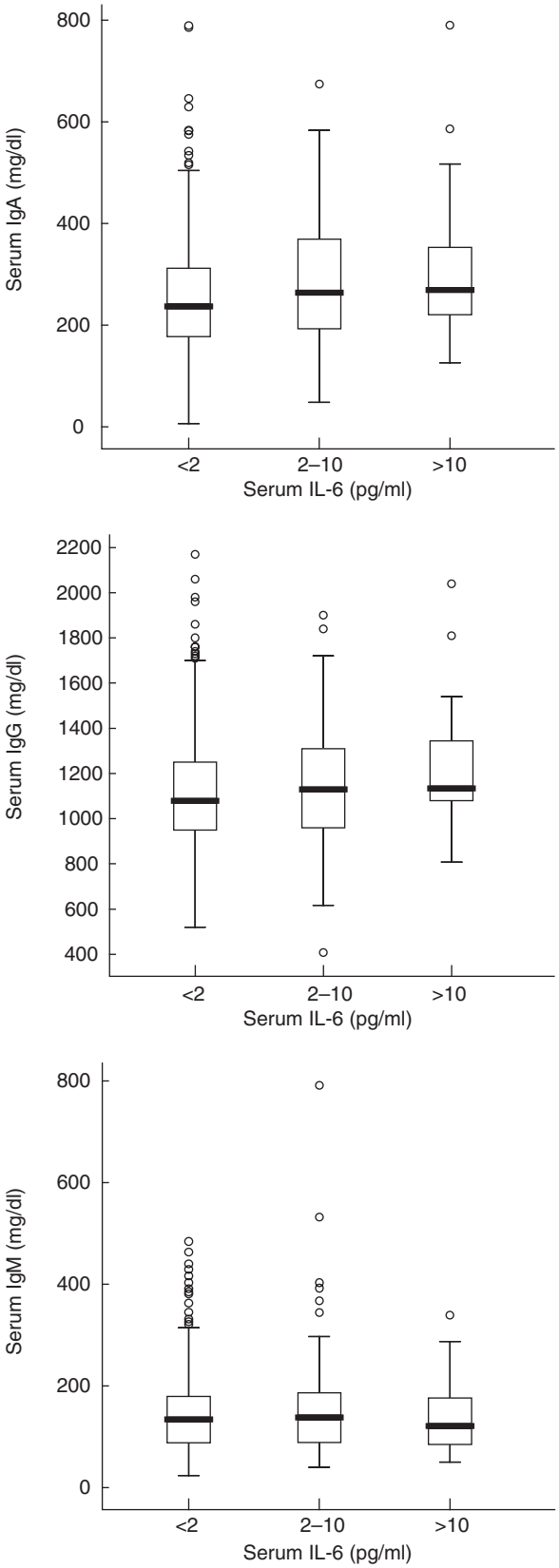

Fig. 5. Serum immunoglobulin (Ig) levels in relation to serum levels of interleukin (IL)-6. Serum IgA and serum IgG tended to increase as the level of IL-6 increased (P for trend = 0·01 and 0·03, respectively).

metabolic syndrome. Serum IgG is associated positively with age and negatively with smoking and moderate drinking. Finally, serum IgM is associated positively with female sex. These features may be important because serum immunoglobulin levels are used commonly in routine clinical practice. The results of the present study cannot be taken as reference values, because the sample represents the general adult population and not 'healthy' individuals [9]. Future studies aimed at defining reference immunoglobulin values should consider partitioning by these factors. Also, these factors should be taken into account when interpreting serum levels of IgG, IgA and IgM.

Acknowledgements

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