

TITLE PAGE**Relationship between Gamma-glutamyltransferase and Fat Mass in a General Population of 8 to 17 Years Old Children. The FLVS II Study.**

Running title: GGT and fat mass in children

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ABSTRACT

Aim:

Elevated gamma-glutamyltransferase (GGT) is positively associated with severity of obesity in obese children and with increased BMI and waist circumference in general populations of adults. We aimed to study the relationships between GGT and anthropometric parameters in a general population of children.

Methods:

This cross-sectional study was conducted in 219 boys and 214 girls included in the Fleurbaix Laventie Ville Santé II study. Weight, height, four skinfolds and waist circumference were measured. We compared the means of GGT activity according to gender and Tanner stage, and according to overweight status (IOTF definition) and quartiles of anthropometric parameters. We then calculated partial Pearson correlations by gender between GGT and anthropometric parameters taking age and Tanner stage into account.

Results:

GGT activity was higher in boys than in girls as soon as puberty started ($p < 0.001$). The higher difference was observed for Tanner stage III (GGT=10.2 UI/L, CI_{95%} of mean [9.5, 11.1] vs 7.8 UI/L [7.2, 8.4]). Anthropometric parameters were significantly associated with GGT, particularly the waist circumference ($r=0.28$ in boys; $r=0.24$ in girls). After an additional adjustment for sum of skinfolds, this correlation disappeared in boys ($r=0.06$), and was still significant in girls ($r=0.19$).

Conclusion:

In a general population of children, overweight and abdominal fat distribution were associated with increased GGT. As some studies have shown that GGT could predict the metabolic syndrome in children and type 2 diabetes in adults, the modest elevation of GGT observed in overweight children may be of pathophysiological importance on the long term.

Keywords: children, epidemiology, gamma-glutamyltransferase, overweight, waist circumference

Titre: Relation entre gamma-glutamyltransferase et masse grasse dans une population d'enfants âgés de 8 à 17 ans. Etude FLVS II.

Objectif:

Une augmentation de la gamma-glutamyltransferase (GGT) est associée positivement à la sévérité de l'obésité chez les enfants obèses et, en population générale, à une augmentation du BMI et du tour de taille chez l'adulte. Notre but était d'étudier la relation entre GGT et paramètres anthropométriques chez l'enfant, en population générale.

Méthodes:

Cette étude transversale a été conduite chez 219 garçons et 214 filles inclus dans l'étude Fleurbaix Laventie Ville Santé II. Poids, taille, quatre plis cutanés et tour de taille étaient mesurés. Nous avons comparé les moyennes d'activité de la GGT selon le sexe et le stade de Tanner, et selon le surpoids (définition IOTF) et les quartiles des paramètres anthropométriques. Nous avons ensuite calculé les corrélations partielles de Pearson par sexe entre GGT et paramètres anthropométriques en ajustant sur âge et stade de Tanner.

Résultats:

Dès le début de la puberté, l'activité de la GGT était plus importante chez les garçons ($p < 0.001$). La plus grande différence était observée au stade de Tanner III (GGT=10.2 UI/L IC_{95%} de la moyenne [9.5, 11.1] vs 7.8 UI/L [7.2, 8.4]). Les paramètres anthropométriques étaient significativement associés à la GGT, en particulier le tour de taille ($r=0.28$ chez les garçons; $r=0.24$ chez les filles). Après un ajustement supplémentaire sur la somme des plis, la corrélation disparaissait chez les garçons ($r=0.06$) et restait significative chez les filles ($r=0.19$).

Conclusion:

Dans une population générale d'enfants, le surpoids et une répartition abdominale des graisses étaient associés à une augmentation de la GGT. Des études ont montré que la GGT pouvait prédire l'apparition du syndrome métabolique chez l'enfant et le diabète de type 2 chez l'adulte. Les conséquences à long terme de l'augmentation modeste de la GGT observée chez des enfants en surpoids restent à évaluer.

Mots-clefs: enfants, épidémiologie, gamma-glutamyltransferase, surpoids, tour de taille

Introduction

In adults, liver steatosis is a well-known complication of obesity (1, 2). It is characterized by an increase in liver enzymes such as transaminases and gamma-glutamyltransferase (GGT) and can evolve into a fibrosis (3).

In liver disease, the mechanism leading to an increase in GGT is not well understood: it is probably not simply due to release of enzyme from damaged cells, but may also be due to a release of the enzyme expressed on the cell membrane more readily than usual (3). Because of its low specificity, GGT is not commonly used alone as a marker of liver function (4). Indeed, enzyme increases with drugs exposure and alcohol intake and is usually rather used as a marker of alcohol consumption or liver disease evolution (3).

A study has shown that steatosis can already occur in obese children (5). The population of children is of interest to study this enzyme within its normal range, because they are mostly free of any drug or alcohol intake. Thus, they offer the opportunity to study the other sources of variability in GGT activity and especially the role of fat mass level and fat mass distribution. Little is known about GGT activity in the general population of children.

The first aim of our study was to provide a description of GGT concentration in boys and girls. The other aim was to assess the correlation between GGT and anthropometric indices of adiposity.

Methods

Population

The children were recruited in the cities of Fleurbaix and Laventie, two neighbour towns in northern France, with respectively 2488 and 4426 inhabitants in 1992, when the Fleurbaix-Laventie Ville Santé study (FLVS) started. The first part of the study, FLVS I, included all children attending primary school in the two cities. It was a five-year follow-up of children involved in a nutritional education program at school and was described elsewhere (6).

The second part, FLVS II, is an epidemiological study on the determinants of weight change. This study was proposed to every family whose children participated in the FLVS I study. We recruited and examined, between April and September 1999, 294 families among the 393 families still living in the two towns (i.e. acceptance rate: 75%). All family members aged 8 years and over (1113 subjects) were examined at home, including healthy girls (251)

and boys (256) aged 8 to 17 years. Written consent was obtained from participants and from their parents for those younger than 18 years. The FLVS II study was approved by the ethics committee (CCPPRB Lille, France) and the computer files comply with French regulations (Loi Informatique et Liberté).

The present study consists of a cross-sectional analysis of the data from the FLVS II baseline examination.

Measurements

Trained physicians collected anthropometric data and determined pubertal stage according to Tanner classification. Weight was measured in light clothes and without shoes to the nearest 0.1 kg, and height to the nearest cm. Waist circumference (WC) was measured to the nearest 0.5 cm, during an expiration, on a normally breathing subject and defined as the smallest diameter between iliac crest and the lower rib. Four skinfolds thicknesses were measured to the nearest 0.1 mm, in duplicate, using Harpenden callipers, on the left side of the body: tricipital (posterior aspect of the arm, at the midpoint between the acromion and the olecranon), bicipital (anterior aspect of the arm, at the midpoint between the acromion and the olecranon), subscapular (1 cm below the inferior angle at the scapula) and supra-iliac (1 cm over the iliac crest, at the midaxillary line). The two values were averaged and the sum of skinfolds was calculated. Body mass index ($BMI = \text{weight}/\text{height}^2$, in kg/m^2) was also calculated and weight status was defined according to the International Obesity Task Force (IOTF) gender- and age- specific BMI cut-off points (7).

A 20 mL blood sample was obtained after an overnight fast and GGT activity was measured at 30°C with an automatic analyser (Hitachi 917, normal range 5-24 UI/L). The serum samples were kept at 4°C and analysed within 48 hours by a single Biochemistry Laboratory, Institut Pasteur, Lille, for all the subjects of the study.

Data analysis

Results are given as mean with 95 % confidence interval of the mean (except in Table 1: mean and 95 % confidence interval of the variable) or percentage with number of subjects. Comparisons between genders were performed with Student's test, Chi-square or exact Fisher tests when appropriate. Parameters were log-transformed when their distribution was skewed, as for GGT activity.

Geometric mean GGT was described according to Tanner stage and gender, and interaction between the effects of Tanner stage and gender was tested.

Adjusted means of GGT activity were calculated according to gender specific quartiles of anthropometric factors (i.e. waist circumference, BMI and sum of skinfolds). For this analysis, we used linear models allowing adjustment for age and for pubertal stages as dummy variables. In the analysis of waist circumference, we additionally adjusted for height.

To assess the associations between anthropometric factors and GGT, we calculated partial Pearson correlation coefficients adjusted for age and pubertal stage. In a second step, in order to test for an association between BMI or WC and GGT independent of subcutaneous fat mass, correlations were additionally adjusted for the sum of skinfolds. In the analysis of waist circumference, we still additionally adjusted for height.

Because of the presence of brothers and sisters in our study population, analysis had to take into account the non-independence between observations from a same family. In that purpose, we introduced a nuclear family variable as a random effect in the linear models (mixed linear model). Partial correlation coefficient calculation had to be adapted as well. Partial correlation coefficient between two variables X1 and X2 is obtained by the Pearson correlation between the residuals of the regressions of X1 and X2 on the adjustment factors including random effect for each family (8).

SAS Software version 8.2 was used for all analyses. Tests were two-sided with alpha-risk equals to 0.05.

Results

A total of 433 children, 219 boys and 214 girls were included in the analyses. Eighteen children declared consuming alcohol at least once a week and were then excluded. Those children were significantly heavier and had higher GGT activity but the differences disappeared after adjustment for age, gender and Tanner stage (data not shown). In addition, fifty-six children were excluded from analyses because of missing measurements (essentially GGT activity in children who refused the blood drawing); those children were younger and after adjustment for age, the other parameters did not differ significantly from the other children (data not shown).

The boys and girls descriptive characteristics are shown in Table 1. Children were on average 13.5 years old and about 10% of them were overweight or obese, without any

significant difference between boys and girls. Girls were more mature than boys ($p=0.02$). Boys were taller than girls ($p=0.001$) and had a higher waist circumference ($p=0.004$); however, mean BMI and sum of skinfolds were significantly lower in boys than in girls ($p=0.04$ and $p<0.0001$ resp.). Mean GGT was higher in boys than in girls (9.7 UI/L vs 8.2 UI/L; $p<0.001$).

Figure 1 shows the GGT activity according to gender and Tanner stage. In boys, GGT increased until Tanner stage 3; whereas in girls a U-shaped relationship was observed (p -interaction between Tanner stage and gender= 0.004). The maximum difference was therefore observed at Tanner stage III (mean GGT=10.2 UI/L, 95% confidence interval of the mean: [9.5, 11.1] for boys and 7.8 UI/L [7.2, 8.4] for girls). Considering this pattern difference, the following analyses were performed separately in boys and girls.

Figures 2-5 represent the mean GGT activity according to the quartiles of anthropometric factors (i.e. waist circumference, BMI, sum of skinfolds and overweight status). In boys, GGT activity increased significantly with increasing quartiles of BMI, waist circumference, sum of skinfolds and overweight status ($p<0.01$). In girls, the associations were weaker and less significant. Interaction between gender and GGT was significant for waist circumference ($p=0.003$) and borderline for BMI ($p=0.07$) and overweight status ($p=0.06$).

For the IOTF definition of obesity, GGT increased from 9.4 UI/L [9.1, 9.8] in normal weight boys to 11.7 UI/L [10.5, 12.9] in overweight or obese boys ($p=0.0002$). In girls, GGT increased from 8.2 UI/L [7.9, 8.5] in normal weight to 9.4 UI/L [8.4, 10.5] in overweight or obese ($p=0.02$).

Table 2 shows partial Pearson correlations between anthropometric factors and GGT separately in boys and girls, when adjusted for age and Tanner stage, and then after additional adjustment for sum of skinfolds. In boys, correlations were very high between the three parameters and GGT ($r=0.27$ to 0.28 ; $p<0.0001$) but correlations with BMI and WC disappeared after adjustment for sum of skinfolds ($r=0.06$; $p=0.4$ for both). In girls, the correlations adjusted for age and Tanner stage were weaker than in boys but significant (BMI: $r=0.19$, $p=0.005$; WC: $r=0.24$, $p=0.0004$; sum of skinfolds: $r=0.15$, $p=0.03$). After adjustment for the sum of skinfolds the correlations persisted with waist circumference in girls ($r=0.19$, $p=0.005$).

Discussion

First, this study showed that GGT activity was higher in boys than in girls as soon as puberty started. Indeed, GGT increased in boys during puberty, whereas the evolution pattern corresponded to a U-shape in girls. Moreover, we showed that anthropometric indices of adiposity were significantly associated with GGT after adjustment for age and Tanner stage. This relationship was stronger in boys than in girls. However, after adjustment for subcutaneous adiposity, there was an independent relationship between abdominal adiposity and GGT in girls only.

The absence of data on other liver enzymes such as the transaminases (alanine aminotransferase, aspartate aminotransferase), used in many studies (9-11) is a weakness of our study. Indeed, elevation of these enzymes would have helped to determine the origin of the GGT increase.

In healthy Korean 10-19 years old population, Park et al (11) have shown a relationship between elevated serum alanine aminotransferase and metabolic syndrome. Association was particularly tight with abdominal obesity. Therefore, this study suggests that an association between markers of liver disorder and anthropometry is seen even with moderate excess weight in healthy children. Therefore, the correlation of GGT with adiposity indices in our population may also be the early consequence of liver exposure to excess fat.

Several previous paediatric studies that have focused on severely obese children have found elevated GGT coexisting with hepatic steatosis (5, 9, 12). One showed that severity of fatty liver (measured by ultrasonographic examination) was positively associated with BMI in children aged between 7 and 18 (9). Median of BMI rose from 28.2 kg/m² (range: 25.4 - 30.8) in children with no fatty liver to 32.5 kg/m² (range: 28.4 - 38.8) in those with severe fatty liver. Similarly to our study, mean GGT was higher in boys than in girls, and waist circumference was the anthropometric parameter most strongly related to the severity of fatty liver. Another study(5) also reported a positive association between steatosis (measured by liver ultrasound scanning) and BMI ($r=0.14$) in 375 obese children. The correlation was even stronger with waist-to-hip ratio ($r=0.36$).

Unlike these studies performed in mostly obese children we did not have any radiological or histological data on children to determine if the increase GGT was associated changes in liver density. However, the modest elevation of GGT observed in our overweight children is unlikely to have radiological consequences. Longitudinal studies could help determine whether it is predictive of the development of hepatic steatosis in children progressing to obesity.

In adults, GGT level is positively associated with BMI (13-17), and abdominal obesity correlates independently (18, 19). Stranges et al calculated partial correlation between GGT and waist circumference adjusted for age and BMI in a 2704 adults sample of New York State. They found that this correlation was higher in women than in men ($r=0.129$ and 0.122 in pre- and post-menopausal respectively vs 0.079 in men) (19). Central body fat distribution – measured as percent body fat adjusted for BMI – was also shown correlated with fatty liver (18). Therefore, obesity, and particularly abdominal obesity may be associated with plasma concentration of hepatic markers even at weak degree of obesity in adults as in children.

A stronger effect of abdominal fat distribution on GGT in females is seen in adult studies as in our children study. As males generally have a central fat distribution, it may explain why the waist circumference no longer contributes to GGT variation, when the amount of fat mass has been accounted for (such as by the sum of skinfolds in our study). A greater heterogeneity of fat distribution in females may explain the persisting independent effect of abdominal fat distribution. It is also possible that other sources of variation of GGT in men (such as alcohol intake) weaken the relationship with abdominal fat distribution in adult males compared to females.

Many prospective publications have shown that GGT (13, 14, 16, 20, 21) or transaminases (10, 22) are predictors of diabetes or the metabolic syndrome in adults and associated with metabolic syndrome recently in children (11). Lee et al. proposed that serum GGT activity might be an intervening factor in the association between obesity and diabetes. Some studies have even shown that GGT predicts diabetes independently of other hepatic markers (13, 14, 20).

GGT is often considered as a marker of liver damage. However it is an ubiquitous enzyme and its induction often occurs as a protective effect against oxidative stress by

regenerating the glutathione (3). Thus, an increase of fat mass and particularly intraabdominal fat mass may lead to increasing oxidative stress (23) and therefore increased GGT. The relationship between plasma GGT activity and oxidative stress may be of pathophysiological importance and explain why GGT has been associated with diabetes onset or fat mass independently of other markers of liver damage (13, 14, 20).

To conclude, this study shows that even in childhood, not only obesity but also overweight and more particularly abdominal fat distribution is associated with increased GGT activity. Whether the increase of plasma GGT activity with central fat mass in children originates mainly from the liver or is also a more general marker of oxidative stress is currently unknown. GGT activity is predictive of development of metabolic syndrome in children and type 2 diabetes in adults. Therefore, the modest elevation of GGT observed in overweight children may be of pathophysiological importance on the long term.

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Tables

Table 1

Physical characteristics¹ and GGT mean values of the study population: gender comparisons

	Boys (n = 219)	Girls (n = 214)	p
Age (years)	13.4 [8.8, 18.1]	13.5 [8.4, 18.7]	0.69
Weight (kg)	48.4 [20.3, 76.5]	47.0 [22.7, 71.4]	0.29
Height (cm)	160.2 [131.0, 189.4]	155.9 [132.1, 179.7]	0.001
BMI (kg/m ²)	18.2 [13.3, 24.8]	18.8 [13.8, 25.5]	0.04
Sum of skinfolds (mm)	33.0 [13.5, 80.5]	45.3 [20.0, 102.6]	<.0001
Waist circumference (mm)	66.8 [52.2, 85.4]	64.6 [52.0, 80.2]	0.004
Gamma GT (UI/L)	9.7 [5.8, 16.0]	8.2 [4.9, 13.9]	<.0001
Tanner stage	1 20 (44)	18 (39)	0.02
	2 21 (46)	12 (26)	
	3 20 (43)	21 (45)	
	4 28 (62)	28 (59)	
	5 11 (24)	21 (45)	
Normal-weight	90.4 (198)	89.3 (191)	0.75
Overweight	8.7 (19)	8.9 (19)	
Obese	0.9 (2)	1.9 (4)	

¹ mean [95% confidence interval of the variable] or percentage (number)

Table 2

Tanner stage and age-adjusted partial Pearson correlations between anthropometric variables and GGT, by gender, with and without adjustment for subcutaneous fat (sum of 4 skinfolds thicknesses)

	Age and Tanner adjusted		Age, Tanner and SK adjusted	
	Boys (n = 219)	Girls (n = 214)	Boys (n = 219)	Girls (n = 214)
BMI	0.27***	0.19**	0.06	0.12
Waist circumference ¹	0.28***	0.24**	0.06	0.19**
Sum of skinfolds (SK)	0.28***	0.15*	.	.

¹Supplementary adjustment for height; * p<0.05, ** p<0.01, *** p<0.0001

Figures

Figure 1

Geometric means GGT and confidence intervals of mean according to gender and pubertal stage (p for interaction with gender=0.004)

Figure 2

Age, Tanner stage and height adjusted GGT activity (and confidence intervals of the mean) in boys and girls, according to gender specific quartiles of WC

Figure 3

Age and Tanner stage adjusted GGT activity (and confidence intervals of the mean) in boys and girls, according to gender specific quartiles of BMI

Figure 4

Age and Tanner stage adjusted GGT activity (and confidence intervals of the mean) in boys and girls, according to gender specific quartiles of SSK

Figure 5

Age and Tanner stage adjusted GGT activity (and confidence intervals of the mean) in boys and girls, according to IOTF-overweight status

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