CORRELATION OF PLASMA LIPOPROTEINS WITH PLASMA PROTEIN GLYCOSYLATION AND INFLAMMATORY MARKERS IN HEALTHY ADOLESCENTS

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BIOTECHNOLOGY

FINAL DEGREE PROJECT

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INDEX

CENTRE DATA

This end-of-degree project in Biotechnology was carried out during my stay at the Pere Virgili Institute for Health Research (IISPV), coordinated by Dr. Elisabet Vilella Cuadrada. It is part of the Department of Medicine and Surgery of the Rovira i Virgili University and is located in the facilities of the Pere Mata Hospital in Reus.

The main objective of this group is the clinical and basic research on the genetic effects and environmental factors in psychiatric diseases; more specifically, the identification of risk factors in different psychiatric diseases. The following studies are currently underway:

- Identification of genetic and molecular markers of the psychiatric diseases (schizophrenia, bipolar disruption and autism)
- Stress implications on mental pathologies
- Evaluation of the cognitive function on persons with personality disruption and other psychiatric diseases
- Evaluation of processes on the psychologist interventions on mental health
- Validation of tools in mental health
- Incipient psychotic disruption: high risk population, first non-affective, affective and critic period episodes.
- Co-morbidity of psychiatric disruptions in people with intellectual disability
- Early onset-schizophrenia of age

ABSTRACT:

Introduction: Psychotic people, characterised by an abnormal condition of their mind that results in difficulties determining what is real and what is not real, usually suffer form metabolic syndrome as a comorbid disease of psychosis. It is believed that this metabolic syndrome leads to cardiovascular diseases which increase the mortality rate of people suffering from this disease. Therefore, the early detection of these metabolic changes is key to halting the progression of these cardiovascular diseases.

Background: Some patterns of the plasmatic lipoproteins fraction, plasma proteins glycosylation and inflammatory state in adults with first episode of psychosis have been described. Despite this, there are only few studies describing these metabolic profiles in adolescents.

Hypothesis: The levels of plasma lipoproteins Very-Low-, Intermediate-, Low- and High-Density Lipoproteins (VLDL, IDL, LDL and HDL respectively) and the different glycation of plasma proteins, GlycA, GlycB and GlycF, directly correlate with the plasma inflammatory markers of Interleukin-6 (IL6) and C Reactive Protein (CRP).

Objective/s:

1. Describe the lipoprotein profile (VLDL, IDL, LDL and HDL), the protein glycosylation profile (GlycA, GlycB and GlycF) and inflammatory markers profile (IL-6 and CRP) in a cohort of healthy adolescents.
2. Measure the correlation between plasma lipoproteins (VLDL, IDL, LDL and HDL) and glycoprotein profile (GlycA, GlycB and GlycF) and inflammatory plasma markers (IL-6 and CRP).

Materials and Methods: Lipoproteins (VLDL, IDL, LDL and HDL), protein glycosylation (GlycA, GlycB and GlycF) and inflammatory markers (IL-6 and CRP) were measured by High-Nuclear Magnetic Resonance (H-NMR) in plasma samples of 31 healthy adolescents (mean age 13 years). Descriptive analysis and variable correlations between the studied parameters were carried out using the PSPP statistical package (free version of SPSS).

Results: In the analysis of correlations within the four groups classified by sex and puberty, we have found numerous positive correlations. We highlight the coincident positive correlations between VLDL and GlycA and GlycF in the groups of boys of mature and immature puberty and between LDL and GlycA and GlycF in those of girls. We also found positive correlations between incomplete puberty groups of both sexes for VLDL and GlycA and GlycB.

Conclusions: GlycA and GlycF probably have an important role in the metabolism during the puberal development, having also a possible proportional relationship with VLDL molecules in the case of boys and with LDL in the case of girls, being these lipoprotein molecules key for the development of each sex.

Key words: lipoproteins, protein glycosylation, inflammation, adolescents.

RESUMEN:

Introducción: Las personas psicóticas, caracterizadas por una condición anormal de su mente que resulta en dificultades para determinar lo que es real y lo que no lo es, generalmente sufren de síndrome metabólico como una enfermedad comórbida de la psicosis. Se cree que este síndrome metabólico conduce a enfermedades cardiovasculares que aumentan la tasa de mortalidad de las personas que sufren esta enfermedad. Por lo tanto, la detección temprana de estos cambios metabólicos es clave para detener la progresión de estas enfermedades cardiovasculares.

Antecedentes: Se han descrito algunos patrones de la fracción de lipoproteínas plasmáticas, la glicosilación de las proteínas plasmáticas y el estado inflamatorio en adultos con un primer episodio de psicosis. A pesar de ello, sólo hay unos pocos estudios que describen estos perfiles metabólicos en adolescentes.

Hipótesis: Los niveles de lipoproteínas plasmáticas de lipoproteínas de muy baja, media, baja y alta densidad (VLDL, IDL, LDL y HDL respectivamente) y la diferente glicación de las proteínas plasmáticas, GlycA, GlycB y GlycF, correlacionan directamente con los marcadores inflamatorios plasmáticos interleucina 6 (IL-6) y la proteína reactiva C (CRP).

Objetivo/s:

1. Describir el perfil de las lipoproteínas (VLDL, IDL, LDL y HDL), el perfil de glicosilación de las proteínas (GlycA, GlycB y GlycF) y el perfil de los marcadores inflamatorios (IL-6 y CRP) en una cohorte de adolescentes sanos.

2. Medir la correlación entre las lipoproteínas plasmáticas (VLDL, IDL, LDL y HDL) y el perfil de glicoproteínas (GlycA, GlycB y GlycF) y los marcadores plasmáticos inflamatorios (IL-6 y CRP).

Materiales y métodos: Se midieron las lipoproteínas (VLDL, IDL, LDL y HDL), la glicosilación de proteínas (GlycA, GlycB y GlycF) y los marcadores inflamatorios (IL-6 y CRP) mediante Resonancia Magnética Nuclear Alta (H-NMR) en muestras de plasma de 31 adolescentes sanos (edad media 13 años). El análisis descriptivo y las correlaciones entre los parámetros estudiados se llevaron a cabo utilizando el paquete estadístico PSPP (versión gratuita del SPSS).

Resultados: En el análisis de las correlaciones dentro de los cuatro grupos clasificados por sexo y pubertad, hemos encontrado numerosas correlaciones positivas. Destacamos las correlaciones positivas coincidentes entre VLDL y GlycA y GlycF en los grupos de chicos de pubertad madura e inmadura y entre LDL y GlycA y GlycF en los de chicas. También se encontraron coincidencias entre los grupos de pubertad incompleta de niños y niñas para las correlaciones entre VLDL y GlycA y GlycF.

Conclusiones: La GlycA y la GlycF probablemente tienen un papel importante en el metabolismo durante el desarrollo puberal, teniendo también una posible relación proporcional con las moléculas VLDL en el caso de los niños y con las LDL en el caso de las niñas, siendo estas moléculas de lipoproteínas clave para el desarrollo de cada sexo.

Palabras clave: lipoproteínas, glicosilación de proteínas, inflamación, adolescentes.

RESUM:

Introducció: Les persones psicòtiques, caracteritzades per una condició anormal de la seva ment que resulta en dificultats per determinar el que és real i el que no ho és, generalment pateixen de síndrome metabòlica com una malaltia comòrbida de la psicosi. Es creu que aquesta síndrome metabòlica condueix a malalties cardiovasculars que augmenten la taxa de mortalitat de les persones que pateixen aquesta malaltia. Per tant, la detecció primerenca d'aquests canvis metabòlics és clau per aturar la progressió d'aquestes malalties cardiovasculars.

Antecedents: S'han descrit alguns patrons de la fracció de lipoproteïnes plasmàtiques, la glicosilació de les proteïnes plasmàtiques i l'estat inflamatori en adults amb un primer episodi de psicosi. Tot i això, només hi ha uns pocs estudis que descriuen aquests perfils metabòlics en adolescents.

Hipòtesi: Els nivells de lipoproteïnes plasmàtiques de lipoproteïnes de molt baixa, mitjana, baixa i alta densitat (VLDL, IDL, LDL i HDL respectivament) i la diferent glicació de les proteïnes plasmàtiques, GlycA, GlycB i GlycF, correlacionen directament amb els marcadors inflamatoris plasmàtics interleucina 6 (IL-6) i la proteïna reactiva C (CRP).

Objectiu / s:

1. Descriure el perfil de les lipoproteïnes (VLDL, IDL, LDL i HDL), el perfil de glicosilació de les proteïnes (GlycA, GlycB i GlycF) i el perfil dels marcadors inflamatoris (IL-6 i CRP) en una cohort de adolescents sans.

2. Mesurar la correlació entre les lipoproteïnes plasmàtiques (VLDL, IDL, LDL i HDL) i el perfil de glicoproteïnes (GlycA, GlycB i GlycF) i els marcadors plasmàtics inflamatoris (IL-6 i CRP).

Materials i mètodes: Es van a mesurar les lipoproteïnes (VLDL, IDL, LDL i HDL), la glicosilació de proteïnes (GlycA, GlycB i GlycF) i els marcadors inflamatoris (IL-6 i CRP) mitjançant Ressonància Magnètica Nuclear Alta (H-NMR) en mostres de plasma de 31 adolescents sans (edat mitjana 13 anys). L'anàlisi descriptiva i les correlacions entre els paràmetres estudiats es van dur a terme utilitzant el paquet estadístic PSPP (versió gratuïta de l'SPSS).

Resultats: En l'anàlisi de les correlacions dins dels quatre grups classificats per sexe i pubertat, hem trobat nombroses correlacions positives. Destaquem les correlacions positives coincidents entre VLDL i GlycA i GlycF en els grups de nois de pubertat madura i immadura i entre LDL i GlycA i GlycF en els de noies. També es van trobar coincidències entre els grups de pubertat incompleta de nens i nenes per a les correlacions entre VLDL i GlycA i GlycF.

Conclusions: La GlycA i la GlycF probablement tenen un paper important en el metabolisme durant el desenvolupament de el cervell, tenint també una possible relació proporcional amb les molècules

VLDL en el cas dels nens i amb les LDL en el cas de les nenes, sent aquestes molècules de lipoproteïnes clau per al desenvolupament de cada sexe.

Paraules clau: lipoproteïnes, glicosilació de proteïnes, inflamació, adolescents.

List of abbreviations:

BMI, body mass index

C, cholesterol

CRP, C-reactive protein

Glyc, glycan

GlycA, glycan A

GlycB, glycan B

GlycF, glycan F

H_W GlycA, height to width ratio of glycan A

H_W GlycB, height to width ratio of glycan B

H_W, height to width ratio

HDL, high density lipoprotein

HDL-C, cholesterol associated to HDL

HDL-P, number of particles of HDL

HDL-TG, triglycerides associated to HDL

HDL-Z, diameter of HDL particles

H-NMR, High-Nuclear Magnetic Resonance

IDL, intermediate-density lipoprotein

IDL-C, cholesterol associated to IDL

IDL-TG, triglycerides associated to IDL

IL-6, interleukin 6

l, large

LDL, low density lipoprotein

LDL-C, cholesterol associated to LDL

LDL-P, number of particles of LDL

LDLPdivHDLP, LDL particles divided bay HDL particles

LDL-TG, triglycerides associated to LDL

LDL-Z, diameter of LDL particles

l-HDL-P, number of large HDL particles

l-LDL-P, number of large LDL particles

l-VLDL-P, number of large VLDL particles

m, medium

m-HDL-P, number of medium HDL particles

m-LDL-P, number of medium LDL particles

m-VLDL-P, number of medium VLDL particles

NonDHDL_P, non-HDL particles

P, number of particles

s, small

s-HDL-P, number of small HDL particles

s-LDL-P, number of small LDL particles

s-VLDL-P, number of small VLDL particles

TG, triglycerides

TotalPdivHDLP, total number of particles divided by HDL particles

VLDL, very low-density lipoprotein

VLDL-C, cholesterol associated to VLDL

VLDL-P, number of particles of VLDL

VLDL-TG, triglycerides associated to VLDL

VLDL-Z, diameter of VLDL particles

Z, diameter

1. INTRODUCTION

1.1. General field of study: metabolic changes in psychotic adolescents

Background: Metabolic syndrome is a common comorbid diagnostic in patients with psychosis and is believed to increase mortality due to cardiovascular disease in people suffering from this disorder. Therefore, premature detection of this metabolic changes to prevent the progress to the cardiovascular disease is challenging. Conventional biomarkers to detect and follow metabolic syndrome are the c-reactive protein (CRP) as inflammatory marker, LDL cholesterol as a lipoprotein marker among others. Recently the H-NMR allowed to study in detail the lipoprotein profile providing new and more sophisticated lipoprotein biomarkers and also the possibility to have the protein glycation (GlycA, Glyc F and Glyc B parameters) levels in the same analysis. Some studies have described this use of this parameters in adult (>18 years of age) patients with a diagnostic of first episode of psychosis, however few data are available in samples of younger adolescents although the psychosis can out brake at these ages.

Problem: Studies of lipoprotein and protein glycosylation using H-NMR in samples of adolescents with a psychosis diagnostic as well as in healthy controls are lacking.

Aim: Into the frame of a larger study we aimed to characterize the pattern of plasma lipoprotein and protein glycosylation parameters obtained by H-NMR and to compare them with conventional clinical diagnostic parameters (LDL and HDL cholesterol and CRP) in a group of healthy adolescents.

Study design (models of study): A group of 31 adolescents (aged from 11-18 years) without metabolic syndrome (defined as normal systolic and diastolic blood pressure, triglycerides, HDL cholesterol and glycemia) and grouped according to the variable completed or non-completed puberty were selected to determine lipoprotein and glycoprotein profiles using H-NMR in samples of plasma. H-NMR was carried out as described (1).

1.2. State of the art:

1.2.1. Psychosis

Psychosis is defined as mental disorders in which the notion of what is real and what is not is lost, in which the perception of reality is altered causing changes in the normal thinking of the patients. Some of the most common symptoms are delusions and hallucinations. In addition, these psychotic episodes may appear more frequently and become more acute, leading to other mental illnesses such as schizophrenia or bipolar disorder.

There are no specific causes of psychosis, but there is known that the environments in which a person develops daily, is a key factor in the development of the disease, such as lack of sleep or use of certain drugs, alcohol or other drugs such as marijuana. Recently, it has also been described

that there is a strong genetic component in the develop of psychosis thanks to studies of family trees that include people with such disorders (2).

Some studies suggest that approximately 20% of the total world population has light psychotic experiences, and about 2-5% develop severe mental disorders characteristic of psychosis. It has been described that about 100,000 new cases of psychosis are reported in the United States each year. Furthermore, this disease affects a large number of people from different social backgrounds and mostly appears in the late stages of adolescence and 25 years of age.

In the case of schizophrenia, is characterized by a serious neurological disorder that persists throughout the patient's life and affects their way of thinking, feeling and behaving. Some of the most common symptoms are delusions, hallucinations, strange behaviour and impaired cognitive ability. The causes of this disease are genetics, daily environment (3) and alterations in the brain and is manifested by acute psychotic episodes. The prevalence of schizophrenia in the population is about 1%; that is, it affects more than 21 million people worldwide and in Spain there are about 600,000 people affected by this disease.

Even though psychosis affects a considerable percentage of the population, few progresses have been made in the analytical diagnosis of this disease.

It has been proven that psychosis is an illness in which the mortality rate is increased (a person with schizophrenia is 2 to 2.5 times more likely to die at an early age compared with a healthy one; which translates into a reduction from 7 to 24 years of their life) (4). The most influential cause of this, is the comorbid diseases that manifest themselves in psychosis. Specifically, these comorbid diseases have been related to changes in metabolism that lead to metabolic syndromes (5). Metabolic syndrome is defined as a combination of obesity, high blood pressure, low high-density lipoprotein cholesterol (HDL-C), high triglycerides and hyperglycaemia. This metabolic syndrome results in having higher risk from suffer other diseases such as cardiovascular disease. About 60% of deaths in psychiatric patients are due to these comorbidities, half of which are due to cardiovascular diseases (6).

It is clear that there is a direct and proportional relationship between metabolic syndrome and psychosis, although what is not entirely clear is which of both is the cause of the other, so more studies are needed (6).

For all this, the detection of molecular biomarkers characteristic of metabolic syndrome, could help in the early detection of the high-risk population and, consequently, provide a better and early treatment to stop the progression of the disease. Thus, the major cause of mortality in psychosis will be reduced and the average life expectancy of people suffering from it will increase.

1.2.2. Comorbidity of metabolic syndromes and cardiovascular diseases in psychosis

Patients suffering from psychosis are prone to suffering from metabolic changes, leading to worst metabolic parameters, and consequently with a higher risk factor of the appearance of metabolic syndromes. This usually leads to the development of cardiovascular diseases in psychotic patients, increasing mortality and, therefore, reducing life expectancy (7).

Obesity and abnormal glucose and lipid metabolism have been linked to increase the risk factor of cardiovascular diseases (7).

Many studies confirm that people with schizophrenia have a higher incidence of developing an impaired glucose metabolism (diabetes) and its prevalence is higher because of the obesity and abnormal glucose levels (8,9). Furthermore, those schizophrenic diabetic patients have been shown to have higher risk factor from suffer cardiovascular diseases (10). One study proved that in diabetes disease a change in the lipoprotein profile occurs, so this relationship between all those risk factors further increases the chances of suffering from cardiovascular disease(11).

As mentioned before, the lipoprotein profile is known to have a strong relationship with the development of cardiovascular disease, specifically abnormal lipoprotein levels, such as LDL or HDL, and also weight and body mass index (BMI) have also been classified as important risk factors (12).

A study with schizophrenic patients showed that they have an increased risk of suffer cardiovascular diseases due to the increased protein glycation leading to an accumulation of ADGE (10). Moreover, cardiovascular diseases have been also linked with inflammation due to the presence of higher inflammatory levels in schizophrenic patients and greater propensity to weight gain, that also affects inflammatory levels (13).

Surprisingly, it has been found that anti-psychotics are likely to aggravate the metabolic profile of the patients in different ways, causing a worsening of the present metabolic syndromes (14,15), even though they are not the direct cause of those metabolic disturbances.

With all these data from previous studies, we can say that weight, BMI, glucose levels, lipoproteins, protein glycation and inflammation, are key factors in the development of cardiovascular disease and, therefore, target molecules for their prediction.

1.2.3. Metabolic characteristics in psychosis

As explained before, psychosis development produces changes in the normal metabolism of its patients, resulting therefore in a mismatched metabolism.

1.2.3.1. Biometric characteristic

It was found that male first episode psychosis patients had a worst general metabolic profile at the beginning of the study (5). In the same study was proved that patients with psychosis have a higher weight and blood pressure after two years of the baseline of them study. In contrast, other study suggests that patients had a lower weight and body mass index (BMI) than control samples, so the evolution of these parameters during the disease is not fully clear (7). One possible explanation

can be that the metabolic profile worsens as the disease progresses and that antipsychotics produce numerous alterations at the molecular level, altering parameters such as weight, BMI or blood pressure (14,15).

1.2.3.2. Inflammation

Young adult patients suffering from schizophrenia and psychosis have higher levels of inflammation and molecular oxidation compared to healthy people (16). One accepted hypothesis for this condition is that psychological stress in psychosis produces an immune response in which there are secreted inflammatory molecules as a stress response. Moreover, there are big differences in the regulation of the inflammatory pathways in adolescents and adults with onset psychosis (13). It has also been proved that there is an increased activity of IL-18 in adolescents with early-onset-psychosis (17). Thus, with all these results we can conclude that psychosis associates with an increased inflammatory response, although not enough longitudinal studies could confirm if inflammation is a cause or a consequence of psychosis.

1.2.3.3. Glycosylation

It has been described that in recent-onset psychotic young adults, the ADGE levels are more elevated caused by an oxidative stress (10). In other study with the same type of subjects shows that the N-glycosylation pattern in serum (peripheral metabolites) and cerebrospinal fluid (central metabolites) changes in patients with schizophrenia and confirms that there are some clear differences in the glycosylation between the schizophrenic male and female group (18). Those results suggest that psychosis produces a change in the glycosylation pattern and that this is influenced by gender.

Moreover, it has been confirmed that in the process of inflammation structural changes in proteins occurs, such as modifications in the levels and patters of glycated proteins. So, we can stablish that the process of glycosylation is directly related with the inflammation state (1).

1.2.3.4. Lipoproteins

It has been observed that young psychotic patients have lower levels of LDL-C, HDL-C and total cholesterol and higher triglycerides levels than healthy subjects (5). Another study showed that the LDL-C and total cholesterol was lower in young psychotic patients than in the healthy controls, but the HDL-C levels were similar as the ones of the control samples (7). Therefore, we can conclude that the progression of the disease causes alterations in the lipoprotein profile.

1.2.4. Biomarkers

The detection of the levels of certain molecules can be a key to the early diagnosis of cardiovascular and metabolic diseases in patients with psychotic illness. These molecules can be

very varied, but we are going to focus on markers of inflammation, lipoproteins and patterns of protein glycation.

1.2.4.1. Inflammatory markers

Even though schizophrenic patients are known to possess higher levels of inflammatory markers, few molecules have been described whose high levels are related to psychosis in adolescents.

One study showed that IL-6 levels in plasma and cerebrospinal fluid were significant higher in recent onset schizophrenic adolescent patients than in the control samples (19). In this same study, the increase in homocysteine and decrease in IL-18R1 in early onset psychosis was also confirmed. Both biomarkers are likely to play a key role in brain immune modulation, neuroinflammation and neurodegeneration in psychosis.

Other study about the differences of the inflammatory pathways' regulation between adults and adolescents with first episode psychosis, highlights an increase in the nuclear transcription factor (NFkB, one of the major regulator of the inflammatory and oxidative cell state), PGE2 and NO2- (soluble inflammatory markers regulated by NFkB) in adolescent patients in contrast to a decrease in the inducible NO synthase (iNOS; pro-inflammatory) in adults (16). These results mean that there are important differences in the development of psychosis between adolescents and adults, of which we can highlight that pro-inflammatory state is higher in adolescents than in adults with a first episode psychosis.

In addition, there was analysed the IL-18, an inflammatory cytokine, level and its binding protein (IL-18BP) in adolescents with early-onset psychosis. There was found higher levels of IL-18 and IL-18/IL-18BP ratios compared with the control ones, what means that there is an activation of the inflammatory pathways during psychosis (17).

Another study established that C-reactive protein (CRP) is a marker of chronic inflammation and is also related to T2DM (type 2 diabetes) /insulin resistance and cardiovascular disease (20).

Moreover, it is known that during the process of inflammation, structural changes in proteins occur, including glycosylation. Thus, the presence of GlycA (N-acetylglucosamine/galactosamine) and Glyc B (sialic acid) has been related to the state of cellular inflammation, which means that they are potential inflammatory markers (21).

1.2.4.2. Glycosylation pattern

One study about the pattern of glycosylation changes in schizophrenia found that there where lower levels of glycated proteins in the unmedicated schizophrenic patients compared with the control samples. Also, it has stablished a difference in this pattern according to the gender, which consist on a higher glycosylation in the schizophrenic males. An example is the 2-fold increase of A4G4LacS4 (a polylactosamine extension of a glycan) in low abundance serum proteins in schizophrenic males, among others (18). Those results show that there is a well determinate pattern of glycosylation in

schizophrenic patients that differs from the pattern of the healthy samples. Also highlight the difference in this pattern according to the gender of the patient.

As I have mentioned earlier, the accumulation of ADGE in schizophrenic subjects causes cardiovascular diseases (10), so these molecules may be a good biomarker of the cardiometabolic risk.

GlycA has recently been described as a marker of depression, although it has only been linked to a long persistence of the disease and not in its early course (22)Haga clic o pulse aquí para escribir texto. associated with T2DM (type 2 diabetes) and cardiovascular diseases (1). Also, as I have mentioned before, GlycA and GycB also are two potential inflammatory markers.

1.2.4.3. Lipoproteins

It has been shown lower levels of HDL-C in schizophrenic patients compared to control samples, so low levels of HDL-C can be considered as an early-onset cardiometabolic risk factor biomarker in schizophrenia (23). Besides, low levels of LDL-C and non-HDL-C have been stablished as the most important biomarkers to predict cardiovascular diseases, being non-HDL-C even further than LDL-C (12).

Despite this, there are many other molecules that affect residually the propensity of CVD and could be good biomarkers of the disease, but they still remain unknown.

All these altered molecular levels are potential biomarkers for the detection of metabolic syndromes and cardiovascular diseases resulting from the psychosis itself. Even so, there are many other molecules involved in the process of disease development that remain unrelated even to this process.

1.2.5. Studies with adolescent subjects

Psychosis is a disease that has been widely studied in adults, but, in spite of being a neurodegenerative disease that generally appears at an early age, there are hardly any studies with young adult subjects and even less with adolescents. Research on this disease in adolescents is key since the development of the disease is not the same in adults as in adolescents as some comparative studies between adults and adolescents have shown, for example, in the regulation of metabolic pathways of inflammation as described in (13).

In the same way, most of the studies carried out in search of potential biomarkers in schizophrenia have been conducted with adult subjects, so studies are needed with adolescents. The reason for this is that, as seen above, there are many metabolic processes that are very different between adults and adolescents. This may be because adolescents go through the process of puberty, which is characterized by multiple hormonal and, therefore, metabolic changes. So, it is logical that there are significant differences between adults and adolescents.

Metabolic differences have also been seen between groups of subjects of different sexes, for example in glycosylation levels.

Therefore, in this study different groups of adolescent subjects will be conducted: according to whether puberty has been completed or not and according to gender. In this way, the results will be more heterogeneous and the differences between groups will be more significant.

Therefore, the intensity of the search for these biomarkers is key to facilitate the detection of these comorbid diseases and to be able to provide an adequate treatment for them.

1.3. Research project of our research group:

The objective of our research group is analysing whether the schizophrenia affects the levels of the inflammatory markers and, therefore, to the content of lipoproteins in plasma and the glycosylation of plasma proteins in adolescent patients. With these results we will establish molecular relationships to clarify the presence of more possible biomarkers in psychosis for a preliminary diagnostic of metabolic syndromes and cardiovascular diseases.

Two different groups will be studied; one formed by patients (adolescent schizophrenics) and the other formed by control subjects (healthy adolescents). Both groups will have the same ethnic characteristics and age, and subgroups will be formed within them according to gender and whether they have completed puberty or not. The results of each subgroup will be contrasted with their corresponding group (patients vs. controls) to analyse the differences in the levels of inflammation markers, lipoproteins and glycoproteins. In this way, it will be possible to obtain more consistent results about the differences according to these two parameters. The final objective will be to analyse the molecular levels according to whether patients suffer from metabolic syndromes and cardiovascular diseases or not and to relate these molecules to the propensity to suffer from these diseases.

This project consists of analysing the control samples made up of healthy teenagers; without any metabolic syndrome neither cardiovascular diseases. To do so, the normal behaviour of these control samples has been analysed and it has been checked if the levels of the inflammation markers can be related to the lipoprotein and glycated protein levels.

This work is the first part of a larger one that is expected to be done in the near future within the research project of the group.

2. HYPOTHESIS:

According to the information a literature cited in the Introduction section we postulate the following hypothesis:

Positive correlations between plasma lipoprotein parameters (VLDL, IDL, LDL and HDL), protein glycosylation parameters (GlycA, GlycB and GlycF) measured by H-NMR, and conventional inflammatory markers (IL6 and CRP) exist in plasma from adolescents selected as not having metabolic syndrome.

3. OBJECTIVES:

To corroborate this hypothesis, we can establish two specific objectives:

- Describe the healthy control samples;
    - biometric parameters,
    - inflammatory markers,
    - plasma lipoprotein profile
    - glycated proteins.
- Analyse and measure the correlations between all these parameters.

4. MATERIALS AND METHODS

4.1. Subjects

The subjects in this study are healthy adolescents (n=31); among them are males and females, aged 11-16 years. All of them are resident in Spain.

The criteria to be followed in order to meet all the requirements of being healthy subjects is not to suffer from metabolic diseases. These requirements are: (1) have a waist circumference above the 90th percentile for age and sex, (2) blood pressure; a systolic blood pressure (SBT) greater than or equal to 130 or a diastolic blood pressure (DBT) greater than or equal to 85, (3) triglyceride level (TG) greater than or equal to 1.7 mmol/L, (4) HDL levels greater than 1.03 mmol/L and (5) glycaemia levels greater than 5.6 mmol/L. To meet the criteria for no metabolic syndrome, the subject must meet the waist circumference requirement and two others.

Patients were excluded if (1) there were no data for their ages, (2) they had levels greater than 10 mg/L for CRP or (3) levels greater than 10 pg/mL for IL-6 (since the latter two indicate high levels of inflammation). In addition to this, there was also carefully analysed their birth history, their parents' history, if they are currently taking any medication, study levels of the subjects and their parents and relatives with any metabolic disease (hypercholesterolemia, hypertension, diabetes and heart disease).

Finally, all the subjects were analysed together and according to their gender and then, in addition, they were classified according to whether or not they had completed the puberty phase.

4.2. Measurement by H-NMR

All lipoprotein (VLDL, IDL, LDL and HDL) and glycoprotein (GlycA, GlycB and GlycF) levels of the plasma samples were measured by H-NMR (high-magnetic nuclear resonance) technique as described (1). Inflammatory markers (CRP and IL-6) were measured by standard clinical methods.

There are many techniques for the measurement of molecules in a plasma sample, but currently nuclear magnetic resonance (NMR) is one of the techniques with the highest performance and specificity. This technique is characterized by a high qualification and molecular quantification of the samples in a short period of time. In addition, this technique offers a complete analysis of the metabolic profile of plasma and serum samples (1). Thus, this technique has been used to perform protein glycosylation and lipoprotein quantification analyses successfully, which leads to considerable clinical advances when analysing the metabolic profile of certain subjects.

Therefore, the frequencies measured by H-NMR (reflected in pulse charts where peaks appear) are used to determine molecular structures and quantify them by means of certain algorithms.

In the case of the measurement of protein glycosylation, the H-NMR technique measures the signal of molecular groups within a global molecule (protein), which is then identified through the signals of the different groups that form it. GlycA represents the signal of the N-acetyl group of N-acetylglucosamine and N-acetylgalactosamine, GlycB, the N-acetyl methyl groups of sialic acid, and GlycF, the acetyl group of N-acetylglucosamine, N-acetylgalactosamine and N-acetylneuraminic bounded glycans in proteins.

4.3. Statistical analysis

The statistical analysis of the results of the samples were processed using the PSPP statistical program (due to the complications caused by Covid-19, there have been limitations to the use of the SPSS (Statistical Package of the Social Sciences) program, so it has been necessary to resort to this free version of the program, which has also limited some of the possibilities of the statistical analysis).

To begin with, we tested all quantitative variables for normal distribution with the X test (p-value>0.05). The variables that did not follow a normal distribution (p-value<0.05) were transformed to log10 or ln and it was checked again if these new variables followed a normal distribution (then histograms of all variables were made to confirm this normal distribution.

After this, the correlation analysis was performed, giving more importance to the correlations between sex and age and lipoproteins and glycoproteins. For continuous variables that follow a normal distribution, the Pearson test was applied and for those that did not, the Spearman test was applied. For categorical variables, the Chi square test was used. Those with p-value<0.05 were considered positive correlations. To confirm these results, scatterplot graphs were made, so that it was illustrated whether a trend was followed among these variables with positive correlations.

Due to the results obtained and explorations carried out, a new key category was created for the division of all subjects, which was the puberty variable. This variable was divided into two categories; 1.00 for subjects who had not completed puberty (covering pre-puberty phases, and phases of pubertal development 2, 3 and 4) and 2.00 for those who had completed it. Thus, four different groups were created based on the categories of puberty and gender.

With this new classification, the mean and standard deviation of all variables were calculated for the different groups and the ANOVA test was performed to compare the variables of two different groups. Values of p-value<0.05 are considered significant. For said significant variables, box charts were made to observe the different trends. Then Pearson and Spearman tests were performed within each group between the different variables to analyse the positive correlations. Finally, the Bonferroni test was applied for these last results.

5. RESULTS

5.1. Sample description

Table 1 shows the biometric and inflammatory markers in the whole sample and in the sample stratified by sex.

The subjects have an age between 11 and 15 years old, which have been classified into two different groups of puberty according to whether they have not completed the puberty phase (prepuberal, pubertal development stages 2, 3 and 4) and those who have. For this variable it must be highlighted its p value of 0.000. For this reason, later all the results are going to be divided according to this variable. There have been described other biometric features of the sample such as weight, height, BMI (body mass index), systolic and diastolic blood pressure and the percentage of body fat. Besides, it was analysed if the subjects have any metabolic syndrome for which no one has any metabolic syndrome.

Table 1. Sample description

	All N=31	**Boys** N=12	**Girls** N=17	**P-value[a]**
Biometric (mean ±SD)				
Age	12.9 ± 1.5	13.4 ± 1.6	12.7 ±1.5	0.600
Puberty 1/2[b] (%)	71.4/28.6	75.0/25.0	68.7/31.3	**0.000***
Weight (kg)	47.0 ± 11.3	50. 6 ± 13.0	44.5 ± 9.5	0.938
Height (cm)	155.3 ± 11.1	158.4 ± 12.0	153.1 ± 10.2	0.990
BMI (kg/m2)	19.2 ± 2.5	19.8 ± 2.9	18.8 ± 2.1	0.916
Systolic blood pressure	102.1 ± 9.5	104.2 ± 11.3	100.7 ± 8.1	0.138
Diastolic blood pressure	56.1 ± 6.8	55.4 ± 6.9	56.6 ± 6.9	0.411
% body fat	22.5 ± 5.1	22.3 ± 6.8	22.6 ± 3.9	0.822
Metabolic syndrome (%)	0	0	0	1.000
Inflammation markers (mean ±SD)				
CRP (mg/L)	0.4 ± 0.6	0.4 ± 0.7	0.4 ± 0.6	**0.001***
IL-6 (pg/mL)	1.8 ± 4.6	3.6 ± 7.0	0.7 ± 1.3	**0.005***

Biometric and inflammatory markers in the whole sample and in the sample stratified by sex.

[a]T-test/Mann Whitney test for continuous variables and Chi squared for categorical variables

[b]Puberty 1=not completed (prepuberal, puberal development stages 2, 3 and 4), 2= completed

*p-value<0.05

There have been analysed two inflammatory markers which are CRP and IL-6. Both parameters have a p value lower than 0.05, with higher levels in the group of boys.

If we look at the table, it does not seem that the CRP is different between boys and girls. A possible explanation may be that, most of the CRP values are concentrated around the value 0.20, but there are subjects (both girls and boys) whose values are not around this range. In the case of boys, these disparate values are significantly greater than 0.20 (around 0.40-0.80) and, in the case of girls, some of their values are around 2.40. So this may be the reason for having obtained a significant result, but which is camouflaged when comparing the averages of both groups. All the described parameters are represented by the mean ± standard deviation and their corresponding p value.

5.2. Lipoprotein parameters

Table 2 shows the lipoprotein parameters in plasma measured using the NMR (nuclear magnetic resonance) in the whole sample and in the sample stratified by sex.

Table 2. Plasma lipoprotein parameters measured using NMR

	All N=29	Boys N=12	Girls N=17	P value[a]
VLDL_C	9.9 ± 3.9	8.5 ± 3.8	11.0 ± 3.7	0.979
IDL_C	4.3 ± 1.8	3.8 ± 1.7	4.7 ± 1.9	0.728
LDL_C	107.3 ± 12.8	108.0 ± 13.3	106.7 ± 12.8	0.746
HDL_C	51.5 ± 8.7	50.4 ± 9.7	52.3 ± 8.0	0.484
VLDL_TG	38.9 ± 10.9	35.4 ± 12.5	41.3 ± 9.3	0.999
IDL_TG	5.8 ± 1.6	5.3 ± 1.7	6.2 ± 1.5	0.998
LDL_TG	9.4 ± 2.9	8.6 ± 3.4	9.9 ± 2.5	0.682
HDL_TG	8.7 ± 3.8	7.4 ± 4.4	9.6 ± 3.1	0.968
VLDL_P	28.6 ± 8.3	26.1 ± 9.3	30.4 ± 7.3	0.990
l_VLDL_P	0.9 ± 0.3	0.8 ± 0.3	0.9 ± 0.2	0.704
m_VLDL_P	3.5 ± 1.3	2.9 ± 1.2	3.9 ± 1.2	0.889
s_VLDL_P	24.3 ± 7.0	22.4 ± 7.9	25.6 ± 6.2	0.813
LDL_P	1042.1 ± 125.4	1055.4 ± 130.2	1032.7 ± 125.0	0.588
l_LDL_P	168.1 ± 21.4	166.2 ± 24.0	169.6 ± 20.1	0.924
m_LDL_P	264.9 ± 66.4	252.4 ± 72.0	273.8 ± 62.9	0.448
s_LDL_P	609.05 ± 64.08	636.93 ± 53.60	589.36 ± 64.9	0.463
HDL_P	25.6 ± 3.5	25.1 ± 4.3	26.0 ± 3.0	0.861
l_HDL_P	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.085
m_HDL_P	8.4 ±1.5	8.1 ± 1.7	8.7 ± 1.3	0.428

s_HDL_P	17.0 ± 2.3	16.8 ± 2.8	17.1 ± 1.9	0.882
VLDL_Z	42.4 ± 0.3	42.3 ± 0.2	42.4 ± 0.3	0.654
LDL_Z	21.1 ± 0.2	21.0 ± 0.2	21.2 ± 0.2	0.897
HDL_Z	8.3 ± 0.0	8.2 ± 0.1	8.3 ± 0.0	0.482
NonDHDL_P	1045.1 ± 129.0	1056.5 ± 132.9	1037.0 ± 129.7	0.685
TotalPdivHDLP	42.3 ± 6.5	43.7 ± 5.2	41.4 ± 7.3	0.819
LDLPdivHDLP	41.2 ± 6.3	42.6 ± 5.2	40.2 ±7.0	0.901

Plasma lipoprotein parameters measured using the NMR (nuclear magnetic resonance) in the whole sample and in the sample stratified by sex.

[a]T-test/Mann Whitney test

*p-value<0.05

The measured parameters are classified into cholesterol (C), triglycerides (TG), total number of particles (P) and number of particles according to the size (s, l and m) and diameter of the particles (Z) and the analysed molecules are VLDL, IDL, LDL and HDL.

Those results do not show any significant p value for the different variables.

5.3. Glycoprotein parameters

Table 3 shows the protein glycation parameters in plasma measured using NMR (nuclear magnetic resonance) in the whole sample and in the sample stratified by sex.

The analysed parameters are Glyc B, Glyc F, Glyc A and then the size of Glyc B and Glyc A.

Table 3. Plasma protein glycation measured using NMR

	All N=29	**Boys N=12**	**Girls N=17**	**P value[a]**
Glyc B	335.3 ± 36.0	326.6 ± 44.9	341.4 ± 28.1	0.595
Glyc F	200.5 ± 20.1	201.6 ± 24.4	199.8 ± 17.2	0.775
Glyc A	641.5 ± 77.2	619.0 ± 84.7	657.4 ± 69.7	0.997
H_W Glyc B	4.2 ± 0.5	4.1 ± 0.6	4.3 ± 0.4	0.597
H_W Glyc A	15.2 ± 1.7	14.8 ± 2.1	15.6 ± 1.5	0.818

Plasma protein glycation parameters measured using NMR (nuclear magnetic resonance) in the whole sample and in the sample stratified by sex.

[a]T-test/Mann Whitney test

*p-value<0.05

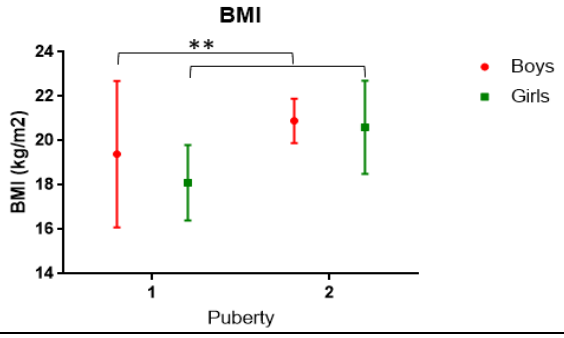
Those results do not show any significant p value for the different variables.

5.4. Effect of puberty on biometric, inflammation, lipoprotein and glycoprotein parameters

Table 4 shows the biometric, lipid and glycoprotein parameters stratifying the sample by puberty and sex. As we have explained before, the parameter of puberty showed a significant value so it will be interesting to analyse all the variables according to this to establish more precise relationships between those different groups.

Regarding biometric parameters we observed that there is a relationship between the age of boys and girls and the two different groups of puberty as it was expected. Also, we can highlight that the BMI is higher is girls with a completed puberty (P=0.024) than girls with a non-completed puberty (Figure 1). Nevertheless, comparing the BMI in the two different groups of puberty of body, there were observed any differences.

Figure 1. BMI levels according to puberty phases and gender



In the group of non-completed puberty comparing boys and girls we observed that girls had higher HDL_TG (P=0.032) and lower s_LDL_P (P=0.034) than boys (Figures 2 and 3). However, in the group of completed puberty no differences between boys and girls were observed.

Figure 2. HDL_TG levels according to puberty phases and gender

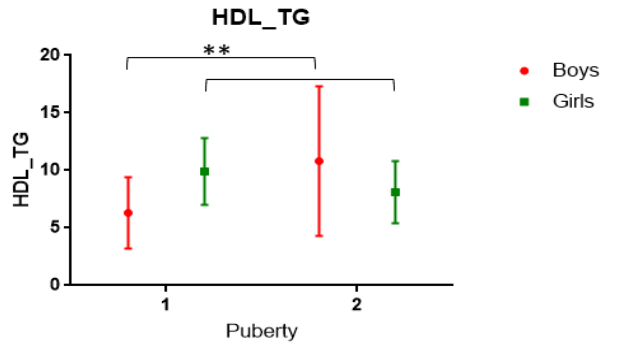
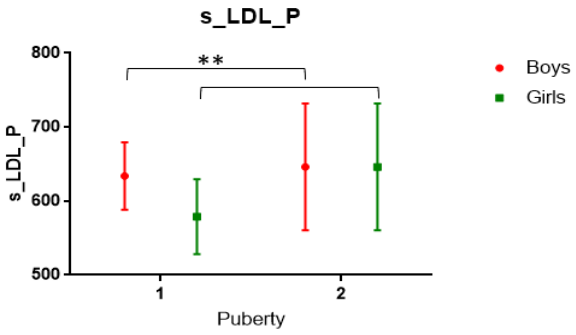


Figure 3. s_HDL_P levels according to puberty phases and gender



When we compared boys with uncompleted puberty to those with completed puberty, we observed that those who have a non-completed puberty have lower levels of VLDL_TG (P=0.037) compared with those one who have a completed puberty. This same difference can also be observed in the case of the VLDL_P (P=0.037) and it is supported by the subgroups of l_VLDL_P (P=0.042) and s_VLDL_P (P=0.036) which are higher in boys with a completed puberty than those with a non-completed puberty (Figure 4). Unlike boys, girls present any differences between the two different groups.

Table 4. Biometric, lipid and glycoprotein parameters stratifying the sample by puberty and sex

	Puberty non completed (1,00)			Puberty completed (2,00)				
	Boys N= 9	**Girls N=11**	**P-value Boys vs girls[a]**	**Boys N= 3**	**Girls N=5**	**P-value Boys vs girls[a]**	**P-value Boys 1 vs 2[a]**	**P-value Girls 1 vs Girls 2[a]**
Biometric (mean ±SD)								
Age	12.6 ± 1.0	12.0 ± 1.1	0.164	15.6 ± 0.5	14.3 ± 0.9	0.075	**0.002***	**0.001***
BMI (kg/m2)	19.4 ± 3.3	18.1 ± 1.7	0.289	20.9 ± 1.0	20.6 ± 2.1	0.838	0.518	**0.024***
% body fat	24.2 ± 6.7	21.8 ± 3.2	0.635	17.2 ± 4.4	25.1 ± 4.6	0.055	0.182	0.114
Inflammation markers (mean ±SD)								
CRP (mg/L)	0.5 ± 0.8	0.5 ± 0.7	0.963	0.2 ± 0.1	0.3 ± 0.2	0.412	0.565	0.602
IL-6 (pg/mL)	4.4 ± 7.9	1.0 ± 1.5	0.656	0.8 ± 1.1	0.0 ± 0.0	0.178	0.834	1.000
Glycoproteins								
Glyc B	328.9 ± 52.2	339.6 ± 31.5	0.135	319.5 ± 8.5	348.6 ± 23.0	0.086	0.856	0.579
Glyc F	194.0 ± 16.7	199.7 ± 17.6	0.710	224.7 ± 33.2	201.0 ± 19.9	0.243	0.077	0.902
Glyc A	607.9 ± 83.6	654.3 ± 81.5	0.094	652.2 ± 96.3	661.5 ± 52.6	0.862	0.268	0.861
H_W Glyc B	4.1 ± 0.7	4.3 ± 0.4	0.137	4.0 ± 0.1	4.4 ± 0.3	0.086	0.854	0.576
H_W Glyc A	14.7 ± 2.3	15.5 ± 1.7	0.093	14.9 ± 1.6	15.8 ± 1.0	0.326	0.481	0.670

Lipoproteins								
VLDL_C	7.2 ± 2.7	10.2 ± 3.6	0.093	12.4 ± 4.6	11.5 ±3.6	0.772	0.051	0.531
IDL_C	3.3 ± 1.1	4.6 ± 1.4	0.090	5.1 ± 2.6	4.1 ± 1.6	0.523	0.158	0.603
LDL_C	106.9 ± 8.4	105.5 ± 10.0	0.552	111.4 ± 25.9	102.6 ± 9.5	0.503	0.739	0.600
HDL_C	49.8 ± 6.4	52.9 ± 9.3	0.517	52.3 ± 18.7	50.1 ± 5.4	0.801	0.795	0.533
VLDL_TG	31.0 ± 8.0	38.9 ± 9.5	0.113	48.8 ± 15.5	45.4 ± 8.8	0.706	**0.037***	0.214
IDL_TG	4.8 ± 1.2	5.9 ± 1.2	0.106	6.6 ± 2.4	6.2 ± 1.5	0.791	0.158	0.698
LDL_TG	7.9 ± 1.8	9.9 ± 2.1	0.054	11.0 ± 6.4	8.7 ± 1.9	0.471	0.229	0.301
HDL_TG	6.3 ± 3.1	9.9 ± 2.9	**0.032***	10.8 ± 6.5	8.1 ± 2.7	0.422	0.167	0.266
VLDL_P	22.8 ± 5.8	28.7 ± 7.3	0.113	36.0 ± 12.0	32.8 ± 7.4	0.646	**0.037***	0.324
l_VLDL_P	0.7 ± 0.3	0.9 ± 0.2	0.282	1.1 ± 0.2	1.0 ± 0.2	0.511	**0.042***	0.214
m_VLDL_P	2.5 ± 0.8	3.6 ± 1.3	0.080	4.0 ± 1.5	4.3 ± 0.9	0.694	0.084	0.261
s_VLDL_P	19.5 ± 4.8	24.3 ± 6.1	0.135	30.9 ± 10.4	27.4 ± 6.5	0.571	**0.036***	0.370
LDL_P	1040.6 ±74.3	1019.6 ± 91.2	0.480	1100.0 ± 259.0	994.3 ± 106.4	0.435	0.596	0.632
l_LDL_P	163.8 ± 16.6	168.2 ± 18.2	0.842	173.2 ± 44.4	163.5 ± 15.1	0.659	0.706	0.626
m_LDL_P	242.9 ±49.2	272.6 ± 53.7	0.348	280.7 ± 131.2	245.0 ± 38.8	0.574	0.555	0.323
s_LDL_P	633.9 ± 45.5	578.8 ± 50.5	**0.034***	646.2 ± 85.8	585.7 ± 75.2	0.335	0.731	0.830
HDL_P	24.6 ± 2.9	26.2 ± 3.4	0.383	26.6 ± 7.9	25.1 ± 2.0	0.702	0.591	0.536
l_HDL_P	0.2 ± 0.0	0.2 ± 0.0	0.443	0.2 ± 0.1	0.2 ± 0.0	0.332	0.281	0.335
m_HDL_P	8.0 ± 1.2	8.9 ± 1.4	0.184	8.5 ± 3.1	8.1 ± 1.0	0.782	0.724	0.260
s_HDL_P	16.4 ± 2.1	17.1 ± 2.2	0.648	17.8 ± 4.9	16.9 ± 1.1	0.670	0.551	0.855

VLDL_Z	42.2 ± 0.3	42.4 ± 0.4	0.450	42.3 ± 0.2	42.6 ± 0.2	0.136	0.854	0.391
LDL_Z	21.0 ± 0.2	21.2 ± 0.2	0.078	21.0 ± 0.3	21.1 ± 0.2	0.683	0.903	0.465
HDL_Z	8.2 ± 0.0	8.3 ± 0.0	0.272	8.2 ± 0.1	8.2 ± 0.0	0.934	0.751	0.137
NonDHDL_P	1038.8 ± 74.9	1022.2 ± 97.2	0.558	1109.4 ± 262.9	1001.9 ± 112.9	0.439	0.528	0.718
TotalPdivHDLP	43.7 ± 5.3	40.8 ± 7.8	0.407	43.4 ± 5.8	41.2 ± 6.7	0.648	0.972	0.930
LDLPdivHDLP	42.8 ± 5.3	39.7 ± 7.4	0.362	42.1 ± 5.8	39.9 ± 6.5	0.644	0.892	0.963

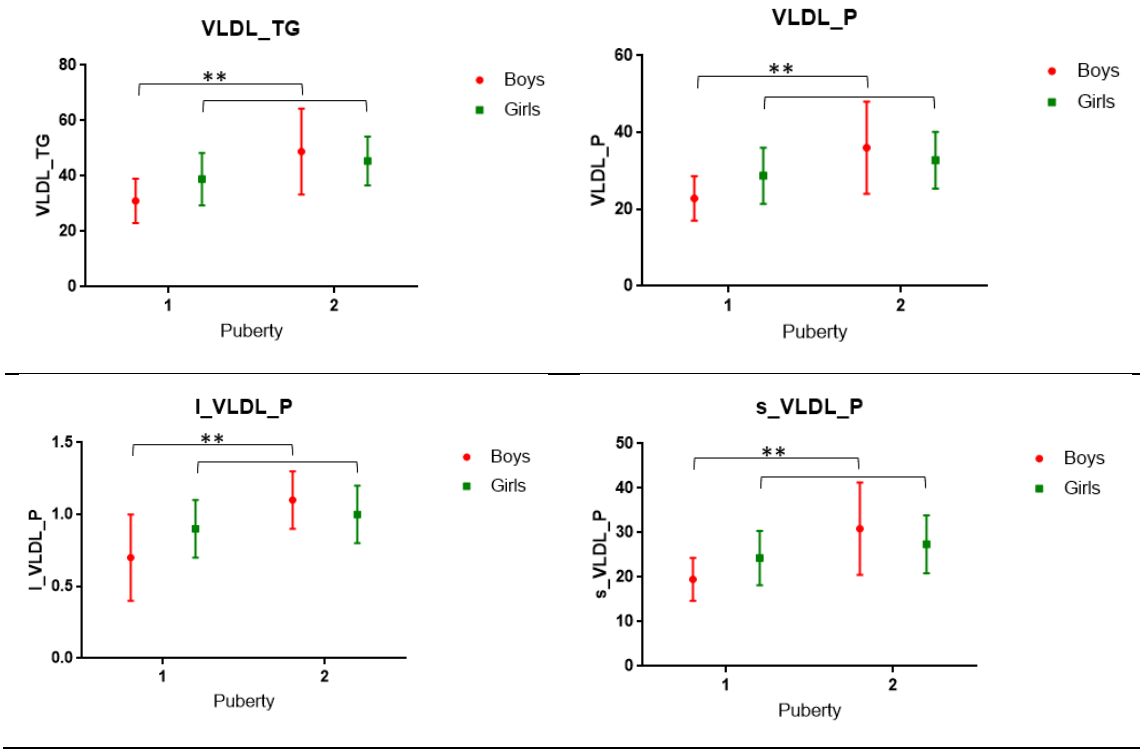
Biometric, lipoproteic and glycoproteic parameters stratifying the sample by puberty and sex.

[a]ANOVA test

*p-value<0.05

**significative p-value after Bonferroni correction

Figure 4. VLDL levels according to puberty phases and gender



5.5. Lipoprotein and glycoprotein correlations in the sample stratified by puberty and sex

We have described the correlations between lipoproteins and glycoproteins according to the different four groups classified by puberty and sex.

Starting with boys with a non-completed puberty, we found that GlycA correlates with VLDL_C (P=0.036), IDL_TG (P=0.029), VLDL_P (P=0.043) and s_VLDL_P (P=0.049) and GlycF with VLDL_TG (P=0.017), VLDL_P (P=0.019), l_VLDL_P (P=0.009) and s_VLDL_P (P=0.013).

In the group of girls with a non-completed puberty, we found that GlycA correlates with VLDL_C (P=0.005), IDL_C (P=0.005), LDL_C (P=0.032), HDL_C (P=0.037), VLDL_TG (P=0.004), IDL_TG (P=0.003), LDL_TG (P=0.040), VLDL_P (P=0.005), l_VLDL_P (P=0.026), m_VLDL_P (P=0.010), s_VLDL_P (P=0.009), LDL_P (P=0.014), NonDHDL_P (P=0.009), TotalPdivHDLP (P=0.005) and LDLPdivHDLP (P=0.005). Furthermore, GlycF correlates with VLDL_C (P=0.024), VLDL_TG (P=0.010), LDL_TG (P=0.047), HDL_TG (P=0.044), VLDLP (0.048), l_VLDL_P (P=0.045), m_VLDL_P (P=0.035) and s_VLDL_P (P=0.018). We have found correlations between GlycB and IDL_TG (P=0.040), TotalPdivHDLP (P=0.025) and LDLPdivHDLP (P=0.026). Also, H_W GlycA correlates with VLDL_C (P=0.010), IDL_C (P 0.011), HDL_C (P=0.050), VLDL_TG (P=0.008), IDL_TG (P=0.006), VLDL_P (P=0.010), l_VLDL_P (P=0.033),

m_VLDL_P (P=0.026), s_VLDL_P (P=0.014), LDL_P (P=0.026), NonDHDL_P (P=0.018), TotalPdivHDLP (P=0.011) and LDLPdivHDLP (P=0.012). And finally, H_W Glyc B correlates with IDL_TG (P=0.041), TotalPdivHDLP (P=0.026) and LDLPdivHDLP (P=0.027).

When we analyse boys with a completed puberty, we saw that GlycA correlates with HDL_C (P=0.008), VLDL_C (P=0.003), VLDL_P (P=0.007), m_VLDL_P (P=0.030), s_VLDL_P (P=0.004) and HDL_P (P=0.012). GlycF only correlates with l_LDL_P (P=0.015). We found that GlycB correlates with LDL_TG (P=0.020), HDL_TG (P=0.010), LDL_P (P=0.013), m_LDL_P (P=0.035) and NonDHDL_P (P=0.009). Also, there have been establish correlations between H_W GlycA and IDL_TG (P= 0.020), s_LDL_P (P= 0.009) and m_HDL_P (0.000). And H_W GlycB correlates with IDLTG (P=0.049), LDL_TG (P=0.037), HDL_TG (P=0.027), LDL_P (P=0.030) and NonDHDL_P (P=0.026). And finally, the group of girls with a completed puberty, where we found that GlycA correlates with LDL_C (P=0.040), LDL_P (P=0.030) and NonDHDL_P (P=0.037) and that GlycF correlates with LDL_TG (P=0.015) and m_LDL_P (P=0.018).

5.6. Correlations with inflammatory markers in the sample stratified by puberty and sex

For both markers of inflammation, some positive correlations have been found: in the group of boys with immature puberty, CRP only correlates with age (P=0.007). In the group of girls with immature puberty CRP correlates with % of body fat (P=0.030), l_VLDL_P (P=0.020) and l_HDL_P (P=0.007), and, in the case of IL-6, with VLDL_P (P=0.048) and s_VLDL_P (P=0.025). And in the group of boys with mature puberty CRP correlates with l_VLDL_P (P=0.020).

5.7. Correlations with biometric parameters in the sample stratified by puberty and sex

In the case of biometric parameters, in the group of boys with incomplete puberty, the age correlates with CRP and m_VLDL_P (P=0. 23) and the % of body fat with IDL_TG (P=0.016), HDL_TG (P=0.022), HDL_P (P=0.016), s_HDL_P (P=0.011), TotalPdivHDLP (P=0.045) and LDLPdivHDLP (P=0.042). In the case of girls with incomplete puberty, the % of body fat correlates with CRP, IDL_C (P=0.036) and LDL_TG (P=0.027). In the group of boys with complete puberty, BMI correlates with VLDL_C (P=0.020) and s_HDL_P (P=0.007). And in the group of girls with complete puberty BMI correlates with LDL_C (P=0.013), LDL_TG (P=0.007), LDL_P (P=0.038), l_LDL_P (P=0. 042), m_LDL_P (P=0.019) and NonDHDL_P (P=0.038), and the % of

body fat with LDL_TG (P=0.010), l_LDL_P (P=0.025), m_LDL_P (P=0.003) and GlycF (P=0.008).

6. DISCUSSION

6.1. Biometric, inflammatory markers, lipoprotein and glycosylation significant results between boys and girls

In this paper we focus on the relationship between glycoproteins and lipoproteins. As I said before, a strong relationship between glycoproteins (GlycA and GlycB) and inflammation (CRP and IL-6) has been described (24), so we are more interested in the relationship of these with lipoprotein levels.

However, it should be noted that the two inflammation markers analysed (CRP and IL-6) show a different trend when their levels are compared according to sex (especially for IL-6) as described also in previous studies (24) with young adult subjects. This difference describes higher levels of inflammation markers for boys compared to girls. Despite these results, in a study with subjects between 47 and 64 years old, no difference in CRP levels was observed when comparing between the two sexes, considering the effect of estrogens on these levels (20). However, this significant difference between the two sexes may be due to a different inflammatory regulation according the sex.

Surprisingly, no difference in lipoprotein parameters has been found between the samples of different sexes, although some difference between them has been previously described; such as a study that observed that HDL_C was slightly higher in women compared to men between 15 and 24 years old (23).

Nor any differences have been observed between different genders in glycoprotein patterns, such as differences in the glycosylation pattern between male and female subjects (18) or associations of higher levels of GlycA and GlycF for females as is described in (20,24). Despite these results in some studies, others do not describe differences for GlycA, GlycB, GlycF, H_W GlycA or H_W GlycB when comparing these levels in young adult men and women (24).

6.2. Influence of puberty on biometric, inflammation, lipoprotein and glycoprotein parameters

If we analyse the existing differences according to the stage of puberty within the same gender group, in the case of boys, we observe slight differences in the trends of different lipoprotein levels of VLDL (VLDL_TG, VLDL_P, l_VLDL_P and s_VLDL_P) (Figure 4). Despite not having obtained significant results for these variables, we can say that VLDL levels, in general, follow a different trend within the group of boys; those who have completed puberty have higher levels than those who have not. Thus, we can say that VLDL could play a key role in the pubertal development within the group of boys.

In the case of girls, a small increase in BMI is observed in the group that has completed puberty, we can speculate that the hormonal changes probably influenced body weight and therefore BMI (Figure 1).

On the other hand, when we study the effect of sex within the same puberty phase group, within the noncompleted puberty group, we find propensities for higher levels of HDL_TG in girls (Figure 2) and lower levels in s_LDL_P (Figure 3) compared to boys. This indicates a possible slightly different metabolism between boys and girls in the puberty phase.

All these results mark slight differences between the groups that are compared although we cannot say that they are very marked differences since they are not totally significant results. Furthermore, as there are no studies that share the characteristics of the subjects that are analysed here, they cannot be contrasted with other results and more accurate conclusions can be drawn from them.

Despite this, with these results we can predict that there is a trend towards a greater molecular change in the different phases of puberty than in sex, which is a factor that is little included in the studies carried out with young subjects, but key to the analysis of the results.

6.3. Glycoprotein and lipoprotein correlations in the sample stratified by puberty and sex

As we can see in the correlation results, in general terms, we can say that among all the parameters analysed in this study, those that present a greater relationship between them are lipoproteins and glycoproteins within the four different groups according to sex and puberty (Annex Table 1), thus establishing the metabolic importance between the different levels of lipoproteins and the patterns of protein glycation in the body. It should also be noted that these relationships mostly present positive r-values, so we have relationships with positive trends between lipoprotein vs glycoprotein levels.

These results cannot be compared with any previous results, since no studies have been conducted on the relationship between lipoproteins and glycosylation pattern in healthy adolescents. Moreover, studies that focus on these molecular groups do not look deeply into different subgroups; for example, studies of protein glycation are mostly directed at GlycA only, while other patterns (such as GlycF) have not been studied at all.

6.3.1. Effect of sex

When we contrast the coincident correlations between the same sexes and different phases of puberty, within the group of boys, we find coincidences for GlycA

(VLDL_P and s_VLDL_P) and GlycF (l_VLDL_P). All these molecules belong to the category of VLDL, so we can say that within the male sex, there is an important metabolic relationship between VLDL and GlycA and GlycF glycation patterns, over which an increase in VLDL levels can be said to be likely related to an increase in GlycA and GlycF glycation patterns.

In the case of the group of girls belonging to the two different groups of puberty, we also found similarities in the correlations with GlycA (LDL_C, LDL_P and NonDHDL_P) and with GlycF (LDL_TG). In this case, all these parameters are included in the LDL category, so that, unlike in boys, in women's metabolism LDL molecules are strongly related to the glycation patterns GlycA and GlycF, so we can predict that higher LDL levels are probably related to increased patterns of protein glycation GlycA and GlycF.

Surprisingly, in both cases the glycoprotein patterns that are related coincide; GlycA and GlycB, so we can deduce that probably both are key molecules in metabolism in the pre- and post-puberty phases.

6.3.2. Effect of puberty

If we analyse the parameters that of correlations that coincide in the same phases of puberty and different sexes, we find coincidences for GlycA (VLDL_C, IDL_TG, VLDL_P and s_VLDL_P) and GlycF (VLDL_TG, VLDL_P, l_VLDL_P and s_VLDL_P) within the group of uncompleted puberty. Interestingly, we found that two of the lipoprotein molecules with positive correlations to GlycA and GlycF match (VLDL_P and s_VLDL_P). In addition, all but one of the molecules that are related to GlycA and GlycF are in the VLDL category. Thus, we can intuit that VLDL molecules are strongly related to GlycA and GlycF type glycosylation in the phases of pubertal development, so an increase in VLDL levels is also accompanied by an increase in GlycA and GlycF glycosylation. This established relationship will probably be marked by the hormonal change experienced at the stage of puberty.

Also note that no matches have been found in the completed puberty group between boys and girls.

Furthermore, with this coincidence within the pubertal development group with GlycA and GlycF, we can say that these are molecules that play an important role in the development phases of puberty and not in post-pubertal metabolism as we had deduced before.

6.3.3. Most significant results

Focusing on the p values between the different variables, it is observed that the most significant values belong mostly to GlycA and H_W GlycA correlations, so we can deduce that the GlycA glycosylation is the most significant among the three Glyc parameters analysed. This indicates that, possibly, it is the most changeable glycation parameter according to the influences of the levels of certain lipoproteins; mainly VLDL and IDL. It is also important to highlight the value of $p<0.001$ and $r=1.00$ between H_W GlycA and m_HDL_P in the group of boys with complete puberty, although due to results of $r=1.00$ for all correlations within this same group, these results are probably so significant due to the low number of subjects in this group (n=3).

6.4. Correlations with inflammatory markers in the sample stratified by puberty and sex

Few positive correlations have been found with the inflammation markers analysed. Most of them are within the group of girls with immature puberty and mainly related to lipoprotein parameters (particles).

No coincidence has been found between groups of different sexes and the same pubertal category or between the same pubertal category and different sexes.

It should be noted that for PRC, in the group of girls, l_VLDL_P has been related to an $r=-1.00$, so there will probably be a trend in which the increase in the level of CRP will be directly proportional to the decrease in the level of the l_VLDL_P. In the case of IL-6, only relationships with VLDL particles in the same group have been found, but with a relationship described by a positive slope between them.

Surprisingly, no correlation has been found between inflammation markers and protein glycation patterns. Previous studies with young adult subjects have confirmed that higher levels of CRP and IL-6 are related with higher GlycA, GlycB, GlycF, H_W GlycA and H_W GlycB levels in healthy and sick subjects (24). This probably indicates that the close relationship between markers of inflammation and glycation patterns is not influenced by sex or by the phases of puberty.

6.5. Correlations with biometric parameters in the sample stratified by puberty and sex

In this case, it should be noted that, in the group of boys with incomplete puberty, positive correlations appear between % of body fat and HDL levels mainly. These are proportionally related, the higher the % of body fat, the higher the HDL levels.

In the group of girls with complete puberty, presents positive correlations between % of body fat and BMI with parameters corresponding to LDL. These results relate the

previous findings of higher BMI for girls with complete puberty and LDL levels for girls, so an increase in BMI will be accompanied by a decrease in LDL levels (the same for % of body fat). Also note that there is a match between both groups of puberty in girls; the % of body fata is related to LDL_TG. This is the only correlation that coincides by comparing the different groups by their categories.

No association with glycation levels, as described in previous papers, has been found; obesity (BMI) was associated with high levels of GlycA strongly and, to a lesser extent, with GlycB, GlycF, H_W GlycA and H_W GlycB in (24).

6.6. Limitations

In general, not many significant differences have been found that lead to the deduction of possible biomarkers within the whole statistical analysis of the samples. This is mainly due to the fact that the subjects have been chosen so that none of them suffer from any metabolic syndrome. Even so we have found some small differences between those who have mature and non-mature puberty and between the two sexes, which, when corrected by Bonferroni disappear, but this perhaps indicates that if the sample were larger these meanings would remain. So even if we have a sample of metabolically healthy adolescents, this technique is subtle and allows us to identify small changes associated with sex (possibly associated with sex hormones) and with puberty (which is also linked to hormonal changes).

Throughout the study we have found some limitations that must be taken into account when analysing the results. The most important of these is the size of the sample; because the number of subjects that have participated in the study is small (n=31), some of the subgroups that we have categorized comprise very few subjects, so the results may not accurately describe their behaviour. Furthermore, when applying the Bonferroni correction, we have lost the meanings we had obtained, probably because of this fact. It should also be mentioned that, in the database where the values of all the variables of the analysed samples of each subject appear, there are lost values (missing) for some variable in certain subjects, so the sample size is even more reduced in the analysis of these variables.

Another limitation could be that in the analysis of the samples some variable that is key in the processes of hormonal change studied has been missed, such as other patterns of glycation or more markers of inflammation.

In addition, differences in molecular levels between subjects of different ethnicities have been described, such as those of inflammation (10), so the inclusion of a greater ethnic variety in the study could also enrich its results.

One of the strengths of this study is the type of subjects that are part of this work; healthy adolescents, in addition to the classification chosen for analysis (gender and puberty). This last category is not usually included as a key variable for the analysis of subjects, when we have observed that there are metabolic differences between mature and immature puberty. To our knowledge, there are no previous similar studies in which the normal metabolic behaviour of healthy adolescents is analysed, and the differences are described according to the stage of puberty they are in. This implies a new perspective of innovative and more complete population analysis.

6.7. Future expectations

Therefore, we can say that this work is a pilot study in which small differences have been found in a small group of subjects, so this may encourage researchers to carry out this same work with a larger number of subjects, in which these differences are appreciated with greater accuracy. Furthermore, it should not be forgotten that this work is part of a larger study in which the results of the samples of schizophrenic patients should be included, comparing both results and determining the existing differences, which will probably be of great clinical help.

7. CONCLUSIONS

Among all the parameters analysed in a first global analysis of the subjects, only the values of inflammation markers (CRP and IL-6) have been differentiated between both sexes, being higher for boys.

As for the analysis between the groups classified according to puberty and sex, a propensity of higher levels of VLDL has been described in boys with complete puberty when comparing the phases of puberty, and the same happens in the case of girls with the variable BMI. Within the phase of pubertal development, a trend of higher levels of HDL_TG in girls and s_LDL_P in boys was observed.

Regarding the analysis of correlations, strong positive correlations between different parameters VLDL and GlycA and GlycF were observed in boys, and of LDL and GlycA and GlycF in girls. Correlations between VLDL and GlycA and GlycF parameters were also observed among incomplete puberty groups. Furthermore, within the same analysis, LDL_TG and % of body fat were found to correlate negatively in the group of girls from different pubertal categories.

All this indicates that the parameters GlycA and GlycF probably have an important role in the pubertal development, having a possible close relation directly proportional with VLDL in the case of boys and with LDL in the case of girls, being both lipoprotein groups key for the individual pubertal development of each sex.

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9. AUTOEVALUATION

First, I have to thank my practices tutor, Elisabet Vilella, who has helped me a lot in the development of the practices during the pandemic period and in the elaboration of this report.

During my internship in the research group of the Pere Mata Institute, I have learned extensively how to perform the statistical analysis of a database of samples from a study. This experience has also helped me to learn to be more autonomous and to work in a different way, compared to what I was used to.

Furthermore, the preparation of this report has helped me to broaden my knowledge of neurodegenerative diseases (mainly psychosis) and certain aspects of the metabolic levels and pathways of the molecules studied.

On the other hand, due to the situation of the pandemic caused by the COVID-19, I have had to carry out most of my training period in a telematic way. This situation has caused limitations when carrying out the practices, such as the use of the free version of the statistical programme SPSS. However, I have been able to obtain a large quantity of results, which I have found difficult to interpret.

Despite this, personally, I am happy with all the work done, all the knowledge I have acquired and the final result of my memory.

10. ANNEX:

10.1. Annex Table 1. Positive correlations within the different groups stratified by puberty and gender

10.1.1. Puberty non completed (1,00) - boys

	Variable	p-value	R
Age	CRP	0.007	0.85
	m_VLDL_P	0.023	-0.78
	VLDL_Z	0.016	-0,81
% of body fat	IDL_TG	0.016	0.85
	HDL_TG	0.022	0.83
	HDL_P	0.016	0.85
	s_HDL_P	0.011	0.87
	TotalPdivHDLP	0.045	-0.77
	LDLPdivHDLP	0.042	-0.77
CRP	Age		
VLDL_C	GlycA	0.036	0.74
VLDL_TG	GlycF	0.017	0.80
IDL_TG	% of body fat		
	GlycA	0.029	0.76
HDL_TG	% of body fat		
VLDL_P	GlycF	0.019	0.79
	GlycA	0.043	0.72
l_VLDL_P	GlycF	0.009	0.84
m_VLDL_P	Age		
s_VLDL_P	GlycF	0.013	0.82
	GlycA	0.049	0.71
HDL_P	% of body fat		
s_HDL_TG	% of body fat		
VLDL_Z	Age		
TotalPdivHDLP	% of body fat		
LDLPdivHDLP	% of body fat		

10.1.2. Puberty non completed (1,00) - girls

	Variable	p-value	R
% of body fat	CRP	0.030	-0.65
	IDL_C	0.036	-0.64
	LDL_TG	0.027	-0.66
CRP	% of body fat	0.030	-0.65
	l_VLDL_P	0.020	-1.00
	l_HDL_P	0.007	0.76
IL-6	VLDL_P	0.048	0.67
	s_VLDL_P	0.025	0.73
VLDL_C	GlycF	0.024	0.67
	GlycA	0.005	0.78
	H_W Glyc A	0.010	0.73
IDL_C	% of body fat		
	GlycA	0.005	0.78
	H_W Glyc A	0.011	0.73
LDL_C	Glyc A	0.032	0.64
HDL_C	GlycA	0.037	-0.63
	H_W Glyc A	0.050	-0.60
VLDL_TG	GlycF	0.010	0.73
	GlycA	0.004	0.79
	H_W Glyc A	0.008	0.75
IDL_TG	GlycB	0.040	0.62
	GlycA	0.003	0.79
	H_W Glyc B	0.041	0.62
	H_W Glyc A	0.006	0.77
LDL_TG	% body fat		
	GlycF	0.047	0.61
	GlycA	0.040	0.62
HDL_TG	GlycF	0.044	0.61
VLDL_P	IL6		
	GlycF	0.014	0.71
	GlycA	0.005	0.77
	H_W Glyc A	0.010	0.74
l_VLDL_P	CRP		
	GlycF	0.045	0.61

	GlycA	0.026	0.67
	H_W Glyc A	0.033	0.64
m_VLDL_P	GlycF	0.035	0.64
	GlycA	0.010	0.73
	H_W Glyc A	0.026	0.66
s_VLDL_P	IL6		
	GlycF	0.018	0.69
	GlycA	0.009	0.74
	H_W Glyc A	0.014	0.71
LDL_P	GlycA	0.014	0.71
	H_W Glyc A	0.026	0.66
l_HDL_P	CRP		
NonDHDL_P	GlycA	0.009	0.74
	H_W Glyc A	0.018	0.69
TotalPdivHDLP	GlycB	0.025	0.67
	GlycA	0.005	0.78
	H_W Glyc B	0.026	0.66
	H_W Glyc A	0.011	0.73
LDLPdivHDLP	GlycB	0.026	0.66
	GlycA	0.005	0.77
	H_W Glyc B	0.027	0.66
	H_W Glyc A	0.012	0.72

10.1.3. Puberty completed (2,00) - boys

	Variable	p-value	R
BMI	VLDL_C	0.020	-1.00
	s_HDL_P	0.007	-1.00
CRP	l_VLDL_P	0.020	-1.00
VLDL_C	BMI		
HDL_C	GlycA	0.008	1.00
VLDL_TG	GlycA	0.003	1.00
IDL_TG	H_W Glyc B	0.049	1.00
	H_W Glyc A	0.020	1.00
LDL_TG	GlycB	0.020	1.00
	H_W Glyc B	0.037	1.00

HDL_TG	GlycB	0.010	1.00
	H_W Glyc B	0.027	1.00
VLDL_P	GlycA	0.007	1.00
l_VLDL_P	CRP		
m_VLDL_P	GlycA	0.030	1.00
s_VLDL_P	GlycA	0.004	1.00
LDL_P	GlycB	0.013	1.00
	H_W Glyc B	0.030	1.00
l_LDL_P	GlycF	0.015	1.00
m_LDL_P	GlycB	0.035	1.00
s_LDL_P	H_W Glyc A	0.009	1.00
HDL_P	GlycA	0.012	1.00
m_HDL_P	H_W Glyc A	0.000	1.00
s_HDL_P	BMI		
NonDHDL_P	GlycB	0.009	1.00
	H_W Glyc B	0.026	1.00

10.1.4. Puberty completed (2,00) - girls

	Variable	p-value	R
BMI	LDL_C	0.013	-0.95
	LDL_TG	0.007	-0.97
	LDL_P	0.038	-0.90
	l_LDL_P	0.042	-0.89
	m_LDL_P	0.019	-0.94
	NonDHDL_P	0.038	-0.90
% of body fat	LDL_TG	0.010	-0.96
	l_LDL_P	0.025	-0.92
	m_LDL_P	0.003	-0.98
	GlycF	0.008	-0.97
LDL_C	BMI		
	GlycA	0.040	0.89
LDL_TG	BMI		
	% of body fat		
	GlycF	0.015	0.95
LDL_P	BMI		

	GlycA	0.030	0.91
l_LDL_P	BMI		
	% of body fat		
m_LDL_P	BMI		
	% of body fat		
	GlycF	0.018	0.94
NonDHDL_P	BMI		
	GlycA	0.037	0.90