TITLE: **Myo-inositol in Down syndrome amniotic fluid. A case-control study.**

RUNNING HEAD: Myo-inositol in Down syndrome amniotic fluid

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BULLETTED STATEMENTS

_ Myo-inositol is involved in metabolic changes that characterize Down syndrome

_ This study shows that Down syndrome metabolic changes occur very early in the fetal life, with amniotic fluid myo-inositol concentrations increasing since the mid-trimester. A possible clinical use of this finding maybe interesting.

Down syndrome (DS) is caused by the trisomy of chromosome 21. In DS, gene products of the extra chromosome 21 may cause abnormal levels of certain metabolites directly or by affecting gene expression of other chromosomes[1]. Myo-inositol (MI) is one of these; it is a polyalcohol deriving from glucose, which is synthesized by a few organs in the body, such as the testes, kidneys and brain[2]. MI concentrations are significantly increased in DS cerebrospinal fluid[1], and its levels are directly correlated with the plasmatic ones[1]. Thus, it is plausible to hypothesize that DS could be associated with MI accumulation not only in cerebrospinal fluid, but also in other fluids, in which it might be detected very early in life. The aim of our study was to evaluate midtrimester amniotic fluid (AF) in DS pregnancies, compared to normal pregnancies.

A retrospective study was carried out on 22 AF samples of DS pregnancies stored at -80°C, collected over the last 6 years, after amniocentesis, performed between 15 and 18 weeks gestation at our Prenatal Diagnosis Centre. A group of 25 samples of normal pregnancy AF,

matched for maternal age, Body Mass Index (BMI) and gestational age, were used as a control group. The protocol was consistent with the principles of the Declaration of Helsinki and all the participants gave their written informed consent for the utilization of 1 ml of their amniotic fluid for experimental purposes. MI quantification was performed by Chelab Pharma Division using gas chromatography-mass spectrometry (GCMS) analysis after extraction with organic solvents and derivation. Injection (1.0 µl) was performed in a split-less mode at 270_C and a capillary column Agilent 122-5532 DB-5 ms (0.25 mm _ 30 m _ 0.25 µm) was used. The total run-time was 15 min: oven at 70_C from 0 to 1 min; 20_C/min to 150_C; 10_C/min to 240_C; 4 min at 320_C post run. The flow rate was fixed at 1.2 ml/min and results were analyzed by an MS 5973 Network Series detector in sim mode. Statistical analysis was carried out with an SPSS statistical package version 17 (SPSS, Chicago, IL). Data are expressed as medians and interquartile range (IQR). The Mann-Whitney test was used to compare AF MI values between groups and Spearman's test was used to correlate normal pregnancy AF values with maternal age, BMI and parity.

The general characteristics of the 2 groups are reported in table 1. There were no statistical differences between them in BMI, gestational age, parity and maternal age. The indications for amniocentesis were maternal age in 17 and positive biochemical genetic screening test in 4. In all DS pregnancies an induced abortion was requested and then performed. A significant difference ($p<0.001$) was found in MI concentrations between groups (figure 1): DS 135.2 µmol/L (IQR 119.2 – 147.8); control group 84.0 µmol/L (IQR 63.1 – 112.8) (figure 1). In the control group there was no correlation between AF MI levels and maternal age ($r = 0.15$, $p = 0.46$); BMI ($r = 0.11$, $p = 0.57$); parity ($r = 0.2$, $p = 0.32$); gestational age ($r = 0.21$, $p = 0.3$) or neonatal weight ($r = - 0.003$, $p = 0.9$).

Inositol is found in the cellular membrane as inositol phosphoglycans (IPGs). The placenta plays a fundamental role in the complex metabolism of inositol. In fact, IPGs are released

into microvilli caveolae[3] by the action of the enzyme glycosylphosphatidylinositol phospholipase D (GPI-PLD) on their lipid precursors, the membrane-associated glycosylphosphatidylinositol (GPI)[4]. Placental GPI-PLD, necessary for the production of IPG, is not produced by the placenta but is taken up from maternal plasma[5]. Placental microvilli have therefore a potential source for placenta-derived IPG molecules which can be released into the maternal blood stream, where they could act as systemic (paracrine) factors, in addition to their role as autocrine mediators of a large number of growth factors and hormones[6]. The lack of correlation between AF MI concentrations and maternal parameters confirms a possible fetal origin of this substance. An increased production of MI AF in subjects affected by DS is in accordance with an accumulation of MI in other DS tissues[7]. In particular, fibroblasts of non-DS patients with Alzheimer disease (AD) did not show enhanced inositol uptake, suggesting that the effect is a specific consequence of the trisomy of the long arm of chromosome 21[7]. Specifically, it has been shown that mental retardation, and after the fourth decade of life, neuropathological and neurochemical changes are similar to those observed in Alzheimer disease. Recent reports of increased MI concentrations in the brain, particularly in the hippocampus, carried out with proton magnetic resonance spectroscopy[8], were in line with previous experiences[9] and further confirm the link between MI concentrations and brain damage. Probably, increased MI concentrations might be linked to the evolution of a defence response to brain damage. Indeed, there is evidence showing that as soon as there is a brain injury, the MI concentration rises[10]. The involvement of MI in brain injury is very likely due to its role as a second messenger of insulin, and insulin at brain level acts as a growth factor closely linked to cognitive and memory function. In animal models, impairment of the insulin signalling system results in a reduction of the cognitive capacity and a reduction of the hippocampal synaptic neurotransmission[11]. All these findings suggest that, because of trisomy, it is very likely that an up-regulation of the MI transporter

gene location on chromosome 21 occurs, and we have demonstrated with this study that the increased MI production occurs very early in pregnancy. Further and larger studies are needed to better clarify the role of MI in DS subjects.

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Table 1: General characteristics of the 2 groups (median and IQR)

	CONTROLS	DOWN'S SYNDROME	P
Maternal age (y)	37.0 (32.5-30.0)	40.0 (34.2-42.0)	0.4
BMI Kg/m²	28.0 (25.2-30.1)	25.5 (19.9-36.1)	0.8
Nulliparous (%)	40.0	36.4	0.7
Gestational age (d)	114 (112-120)	115 (112-123)	0.5

y = years BMI = Body Mass Index % = percentage d = days

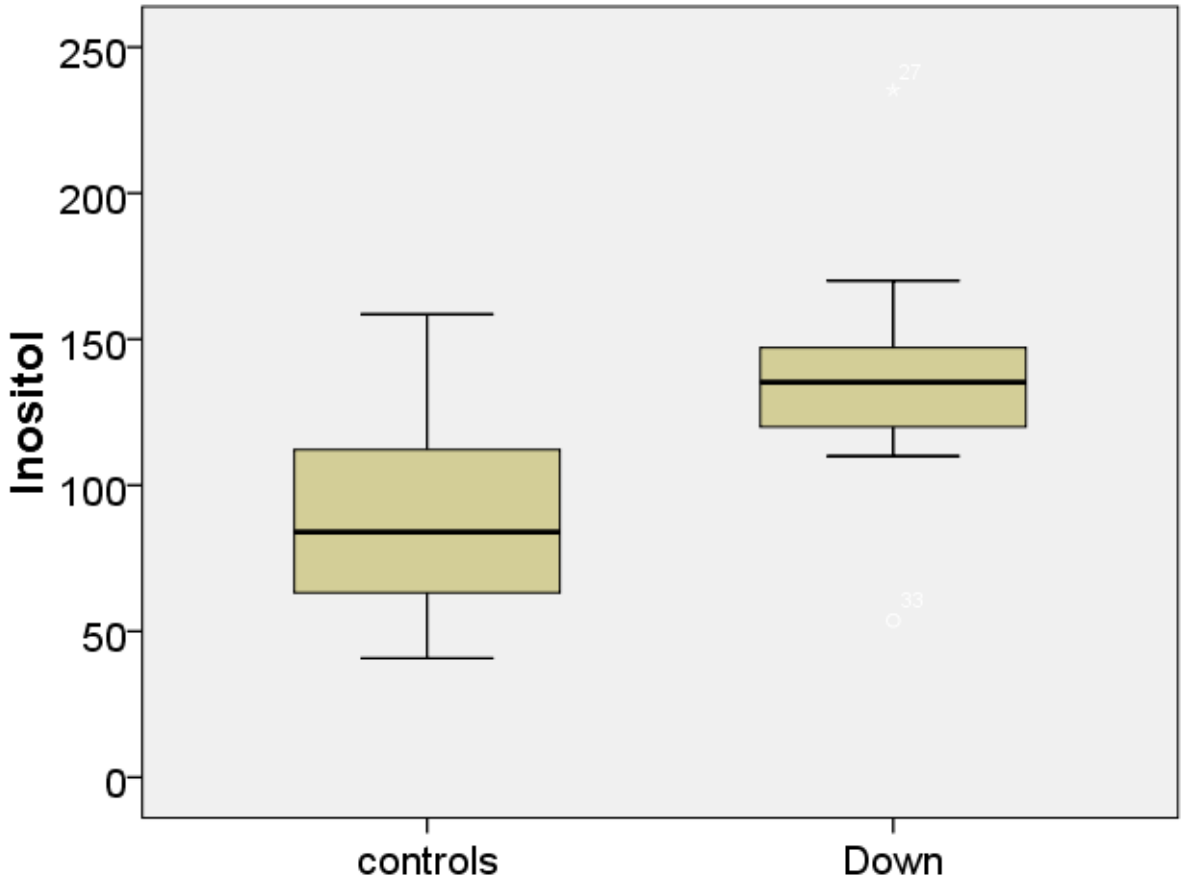

Figure 1: Myo-inositol concentrations (µmol/L) in both groups amniotic fluid