subjects (n=194). Enteroviral RNA was assessed using a highly sensitive reverse transcriptase- polymerase chain reaction method.
Results: The frequency of positive signals corresponding to enteroviral sequence amplification was higher in newly diagnosed T1DM children (9/34, 26.5%) and islet autoantibody-positive first-degree relatives (5/32, 15.6%) than in their corresponding matched controls (2/68, 2.9%, p= 0.0007 and 0/64, 0.0%, p=0.0033, respectively). The presence of enteroviral RNA appeared to be associated with severe diabetic ketoacidosis at onset (pH<7.1, p=0.0328)) and high ICA titres (≥ 20 JDF units, p<0.05).
Conclusion: Despite there is a high circulation of enteroviruses and a low type 1 diabetes incidence in the Cuban population, the presence of enteroviral RNA is associated with type 1 diabetes and β-cell autoimmunity similarly to European countries in which this scenario is inverted.
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0360

Enterovirus genomes in the blood in children with T1DM at disease onset and one year later
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Background and Aims: Enteroviral (EV) infections have been proposed as one triggering environmental factor leading to T1DM. The mechanism(s) through which EV collaborate to beta cell destruction is still unclear. Human EV have long been known as agents of acute diseases, chronic infections being rarely documented. Objectives and hypotheses: To assess whether T1DM were associated with chronic EV infection, we evaluated the presence of EV RNA genomes both at the time of clinical onset and one year later.
Materials and Methods: Blood samples of 68 T1D patients (38 boys; age 2–18 years) have been investigated. Detection of EV genomes was performed by RT-PCR using different primer pairs directed to conserved genomic regions. Sensitivity of the amplification methods in use was 10 genome equivalents per reaction tube. Direct sequencing of purified amplicons allowed identifying EV at the species level.
Results: At onset, EV genomes were detected in 46/68 patients (67%). No viral genomes were detected in 18 healthy control children. Direct amplicon sequencing showed that viruses of the HEV-B and HEV-C species were particularly prevalent. Retesting positive patients one year after clinical onset showed that EV genomes were present in only 1/46 children.
Conclusion: The results confirm the temporal association of EV infection with the onset of T1DM. We were unable to show persistent EV infection in the blood of most pediatric patients. This, however, does not exclude chronic infection of pancreatic islets.

0361

Epigallocatechin 3-gallate protects pancreatic Ins-1 cells against 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced acute toxicity
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Background and Aims: The increasing prevalence of type 2 diabetes prompted several scientist to suggest that environmental contaminants, in addition to obvious nutritional habits, could increase the risk of diabetes development. In this context, dioxins are considered the only environmental contaminants so far identified that could exert widespread effects in the general population. Several epidemiological studies have demonstrated a positive correlation between dioxin exposure and glucose metabolism disorders including diabetes. In order to clarify the biological basis of this correlation we have previously demonstrated that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is highly toxic for INS-1 cells and that, even at very low doses, markedly impairs glucose-stimulated insulin secretion. The aim of this research has been to investigate the protective effect of the green tea polyphenol epicallocatechin 3-gallate (EGCG) against the TCDD-induced toxicity in INS-1 cells.
Materials and Methods: INS-1 cells were exposed for 1 hr to TCDD alone or in the presence of different concentrations (0–200 μM) of EGCG. We have then assayed: 1) cell survival; 2) the morphological alterations by electron microscopy; 3) the mitochondrial membrane potential by cytofluorimetry; 4) the variations in the intracellular calcium concentration by fluo-3/AM-based fluorimetry; 5) glucose-stimulated insulin secretion.
Results: Our results show that EGCG was able to significantly increase the survival of INS-1 cells after 1 hr incubation with TCDD. This protective effect was clearly dose-related and was evident, and quantitatively remarkable for the higher TCDD doses (cell survival was 2.5- and 6-fold higher in 200 μM EGCG-treated cells for 25 and 50 nM TCDD, respectively; p<0.01). The ultrastructure of TCDD-exposed INS-1 cells was also well preserved in the presence of EGCG. One of the most interesting consequences of TCDD treatment was a remarkable activation of autophagy, indicated by the presence of several large autophagic vacuoles containing identifiable organelles, such as mitochondria or degraded membranes. In EGCG-protected cells no autophagic vacuoles were found and interstingly numerous apoptotic bodies were evident in the cytoplasm of surviving cells. EGCG had no effect on the TCDD-induced increase in the intracellular calcium concentration, but partially prevented the TCDD-induced mitochondrial depolarization. EGCG was also able to prevent completely the TCDD-induced impairment of glucose-stimulated insulin secretion.
Conclusions: Our research clearly shows that EGCG is highly effective in preventing the TCDD-induced toxicity in INS-1 cells, in terms of both cell survival and preservation of their physiological properties. This protective effect could be related to the reported inhibitory effect of EGCG on the Aryl Hydrocarbon Receptor (AhR). EGCG indirectly inhibits AhR by binding the accessory protein Hsp90 and thus probably blocking the TCDD-induced activation of the MAPKs signal transduction pathway.
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0362

A cross-sectional study of the association between persistent organic pollutants and glucose intolerance and insulin
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Background: Experimental evidence supports the hypothesis that persistent organic pollutants (POPs) may cause type 2 diabetes. However, epidemiological studies do not convincingly support an association. The Inuit population in Greenland, which is highly exposed to POPs due to a high intake of sea mammals and fatty fish, has experienced a rapid increase in diabetes prevalence over the last 30 years. Thus the aim was to study the association between POPs and diabetes and impaired glucose regulation in a population-based design.
Methods: From 1999 to 2002 the Greenland population study was carried out among adult Inuit living in Greenland. The examination included a 75g OGTT, anthropometric measurements, a structured interview, and blood tests. P-glucose, s-insulin, lipids were analysed, and PCB153 and p,p'-DDE were analysed in a sub-group as biomarkers of POP exposure. Associations were adjusted for age, sex, waist circumference, HDL cholesterol and triglycerides.
Results: Data on POPs were available on 692 individuals, 305 men (mean age 50 years) and 387 women (mean age 49 years). The table shows mean levels of POPs (geometric means) according to glucose tolerance status. (Tab. 1)

Tab. 1

	N (%)	PCB153 ng/g lipid	Mean PCB153 ng/g lipid, adjusted	*p-value*	p,p'-DDE ng/g lipid	Mean p,p'-DDE ng/g lipid, adjusted	*p-value*
Diabetes	71(10.3)	777	752	0.63	1494	1445	0.52
Non-diabetes	621(89.7)	653	722		1240	1358	
IGR	254(36.7)	774	731	0.82	1443	1361	0.90
Non-IGR	438(63.3)	600	722		1153	1370	