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Whole body cholesterol metabolism is impaired in Huntington's disease

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ABSTRACT

We previously reported impaired cholesterol biosynthesis in rodent Huntington Disease (HD) models and HD patients' fibroblasts and post mortem brains. We also found that plasma levels of 24S-hydroxycholesterol (24OHC), the brain specific elimination product of cholesterol considered a marker of brain cholesterol turnover, were significantly reduced in HD patients at any disease stage. In the present study we analysed by mass spectrometry the *fasting* plasma levels of cholesterol, its biosynthetic precursors lanosterol and lathosterol, of the whole-body elimination products 27-hydroxycholesterol and of brain 24OHC in a cohort of premanifest and HD patients at different disease stages. We found that the cholesterol precursors lanosterol and lathosterol (both index of whole body cholesterol synthesis), the levels of the bile acid precursor 27-hydroxycholesterol, and of the brain specific 24OHC, were all significantly reduced in manifest HD patients, suggesting that whole-body and brain cholesterol homeostasis are both impaired in HD.

Huntington's disease (HD) is an inherited autosomal dominant neurodegenerative disorder caused by a CAG triplet repeat expansion in the gene encoding the huntingtin protein (HTT) [11,28]. The mutation results in an elongated stretch of glutamine residues located in the NH_2-terminal of HTT. Wild-type HTT is widely expressed in most tissues and is considered to be involved in several brain functions, including protein trafficking, postsynaptic signalling, and transcriptional regulation. The expanded polyglutamine tract confers a novel toxic function to HTT but loss of function of the wild-type protein might contribute to neuronal dysfunction and death [27–29]. Neurodegeneration of the striatum and the cortex is a pathological hallmark of HD [27,28]: brain MRI volumetric scans show progressive striatal and cortical atrophy [1,10].

We previously reported that the expression of genes involved in cholesterol biosynthesis was reduced in striatum and cortex from R6/2 mice, in human HD brain samples and in fibroblasts [21]. Subsequent studies showed that brain cholesterol synthesis, accumulation, steady state levels and turnover were significantly reduced in brain samples across several rodent models of HD [24–26].

Cholesterol is an essential structural and regulatory component of cell membranes. It is involved in the maturation of CNS, signal transduction, neurotransmitter release, synaptogenesis and membrane trafficking [7]. De novo synthesis and uptake from circulating lipoproteins cover the cholesterol needs of the cells [7]. Almost all the mammalian cells are able to synthesize cholesterol and to express the sophisticated and energy demanding enzymatic machinery required for the de novo synthesis. The cholesterol synthesis is a complex pathway involving several steps and more than 20 enzymes. It begins with the synthesis of mevalonate from acetyl-coenzyme A, proceeds with cyclization of squalene to give the parental steroid, lanosterol and through many steps involving also the formation of lathosterol, ends with cholesterol [6]. Cholesterol represents as much as 2–3% of the wet weight of the brain and about 25% of the whole body cholesterol is located there [6,7]. The majority of brain cholesterol resides in 2 pools: one is represented by the myelin sheaths (oligodendroglia), the other one by the plasma membranes of astrocytes and neurons [21]. De novo synthesis is responsible for almost all cholesterol present there since the blood–brain barrier (BBB) efficiently prevents uptake from the cir-

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Table 1
Demographic data of controls, pre-manifesting and manifesting HD patients.

	Pre-HD ($n=42$)	HD1 ($n=48$)	HD2 ($n=44$)	HD3–5[a] ($n=35$)	Controls ($n=134$)
Female/male	24/18	16/32	23/21	17/18	68/67
Age at exam (years)	33.4 ± 8.1 (18.8–54.5)	47.6 ± 10.8 (25.6–69.8)	52.9 ± 11.6 (27–75)	57.1 ± 12.3 (34.1–81)	48.2 ± 14.6 (21.1–75.8)
Age at onset (years)	–	42.7 ± 9.9 (23.6–66.8)	46.9 ± 10.9 (24–68)	47.8 ± 11.9 (28.1–73.4)	–
Disease duration (years)	–	5.1 ± 4.3 (1–22)	6.3 ± 3.3 (1.7–16)	9.3 ± 4.8 (2–19)	–
TFC	13 ± 0 (13–13)	11.9 ± 0.8 (11–13)	8.2 ± 11 (7–10)	3.9 ± 4.8 (0–6)	–
UHDRS motor score	0.5 ± 1 (0–3)	20.9 ± 12.9 (3–69)	32.7 ± 12.7 (14–58)	52.1 ± 20.7 (11–90)	–
Expanded allele (number of CAG repeats)	43.6 ± 2.6 (39–49)	44.5 ± 3.6 (40–58)	44.5 ± 3.7 (40–55)	45 ± 4.8 (39–68)	–
Probability of disease onset within 5 years	0.06 ± 0.14 (0–0.73)	–	–	–	–
Medication (%)	12	64.5	81.8	85.7	0
SRRI (%)	3	31.2	47.7	20	0
Neuroleptic (%)	0	31.2	52.2	74.2	0
Benzodiazepine (%)	4.8	29.1	29.5	31.4	0
Antioxidant (%)	4.8	10.4	9	0	0
Antiepileptic (%)	0	10.4	6.8	17.1	
Other (%)	11.9	31.2	45.4	48.5	0

Data are presented as mean ± standard deviation (min–max). Pre-HD = pre-manifesting HD gene positive subjects; HD patients are divided in three groups in agreement with TFC scale (HD1 = TFC 11–13; HD2 = TFC 7–10; HD3 = TFC 3–6; HD4 = TFC 1–2; HD5 = TFC 0).

[a] HD3 = 26; HD4 = 6;HD5 = 3.

TFC = total functional capacity; UHDRS = Unified Huntington's Disease Rating Scales; SSRI = selective serotonin reuptake inhibitors. No cholesterol synthesis inhibitors in use by any participant.

culation. Neuronal cells have been found to be able to synthesize cholesterol during the embryogenesis and the early life, but the rate synthesis is dramatically reduced in the adults brain. It has been hypothesised that during postnatal development the neurons down-regulate their own cholesterol synthesis and rely on delivery of cholesterol from astrocytes. The "outsourcing" of cholesterol synthesis may allow neurons to spare the energy for ATP-driven membrane potential [18].

For maintenance of brain homeostasis, cholesterol is converted by the neuronal specific cholesterol 24-hydroxylases (CYP46) into the more polar 24S-hydroxycholesterol (24OHC) and released from the brain into circulation. Plasma 24OHC is almost all of cerebral origin and it is considered an indicator for brain cholesterol homeostasis [2]. In a previous study, we found that plasma levels of 24OHC were significantly reduced in HD patients compared with healthy subjects. The decrease in plasma 24OHC paralleled the reduction of caudate volumes at MRI investigation [14], suggesting that the observed reduction of plasma 24OHC might reflect the progressive neuronal loss in the grey matter, albeit concomitant reduction of brain cholesterol biosynthesis could not be excluded [14].

Since brain cholesterol synthesis cannot be safely measured in humans, in the present study we performed an *in vivo* analysis of cholesterol metabolism in HD. We measured in fasting plasma the levels of cholesterol and the cholesterol precursors lanosterol and lathosterol, considered as surrogate markers for whole body cholesterol synthesis [13]. We also assessed the levels of 27OHC and 24OHC, regarded respectively as markers of cholesterol elimination from extra-cerebral tissues [8] and from the brain [2].

A total of 168 HD mutation-carriers and 133 healthy age-matched controls (spouses or carers), were enrolled in the study. HD subjects were recruited in six different Neurological Italian Centres: Milan (87 subjects), Naples (21 subjects), Isernia (18 subjects), Rome (14 subjects), Florence (14 subjects), Genova (14 subjects). For all participants, blood samples were collected after an overnight fast in agreement with the current procedures for studies on lipid metabolism [3,17,22]. All eligible participants received verbal and written information about the study and signed an informed consent form, according to the Declaration of Helsinki. For symptomatic HD patients with severe cognitive impairment the informed consent was obtained from his/her relative. The study protocol was approved by the Ethics Committees of the Besta Neurological Institute and the other participating Institutions. All patients were evaluated by a standard neurological examination. Detailed family history, age of symptoms onset, medications and other relevant clinical information were collected. Clinical assessment of motor symptoms and total functional capacity (TFC) were determined using the Unified Huntington's Disease Rating Scale (UHDRS). Disease stage was determined according to Marder's specifications [15]. The age at onset was considered the time when motor symptoms were first noticed. Patients with only psychiatric manifestations were excluded from the study.

The cholesterol precursors lathosterol, lanosterol, the bile acid precursor 27OHC and the brain derived 24OHC were measured by isotope dilution mass spectrometry [14,18].

Continuous data were inspected and tested to determine whether distributions were normal by Kolmogorov–Smirnov normality test and compared using Kruskal–Wallis test for non-parametric data or ANOVA with the Scheffé post-test for parametric data, with Holm–Sidak method for All Pirewise Multiple Comparison. Values for statistical significance were set at $P<0.05$. Correlations were computed using Pearson's coefficient. All analyses were performed with Sigmastat 3.01 (Sigma–Aldrich, St Louis, MO, USA).

Clinical, demographic data and medication records of controls, pre-manifesting and manifesting HD patients are summarized in Table 1. None of the study participant was under therapy with cholesterol synthesis inhibitors (i.e. statins).

Cholesterol levels in late stage HD3–5 patients were significantly reduced when compared to HD1 and HD2 patients ($P=0.002$ for both) (Table 2)

The levels of lathosterol were significantly reduced in pre-manifest HD subjects (PreHD) ($P=0.003$), HD2 ($P=0.001$) and HD3–5 ($P=0.003$) when compared to controls. A similar significant reduction in lanosterol levels was found in HD1 ($P=0.005$), HD2 ($P<0.001$) and HD3–5 patients ($P<0.001$). In HD2 and HD3–5 patients, lanosterol was also significantly reduced when compared to PreHD ($P=0.006$).

27OHC concentrations were significantly reduced in preHD, HD1, HD2 and late stage patients compared to controls ($P<0.001$ for all). Late stage patients had 27OHC significantly reduced compared to PreHD ($P=0.003$) and HD1 ($P=0.004$).

The concentration of 24OHC was significantly reduced in HD patients at all disease stages when compared with Controls