General introduction
This chapter provides an overview of the early literature concerning creatine. The reader is referred to chapter 4.2 for an up to date review on creatine, the creatine transporter and creatine transporter deficiency.

1.1 A SHORT HISTORY OF CREATINE

Creatine was first isolated from commercial beef-tea ("bouillon de la Compagnie Hollandaise") in 1832 by Michel Eugène Chevreul who named this new substance after the Greek word for flesh, χρέας. Creatine appeared as clear crystals formed as rectangular prisms with a pearl shine and consisted of oxygen, hydrogen, carbon, and nitrogen in proportions which were not yet determined. The discovery was a mere detail in the life of this famous French chemist, who was born in 1786 in a family of surgeons. He discovered the fatty acids, elucidating the true nature of soaps and inventing the bright burning non-odorous stearin candle. He formulated the laws of contrasting colors, bringing brightness back to the tapestries of the Gobelins and inspiring the impressionist painters. He disproved a supernatural cause of the magic pendulum and divining rod and, in his nineties, became a pioneer in gerontology. While he grew up during the French revolution, he received congratulations on his 100th birthday from Queen Victoria and was the first to appear in a photo-interview before he died April 9th 1889, aged 102 years, after succumbing on his return from his daily drives to see the progress made in the erection of the Eiffel Tower.

Chevreul, at the age of one hundred years, photographed by Paul Nadar, 1886.
The history of creatine continued with the German Justus Liebig, who confirmed the presence of creatine in the muscle of several animals in 1847 and noted that a fox shot during the hunt contained ten times more creatine than a fox living in captivity. He also described the formation of creatinine from creatine and extracted both substances from human urine (as did Pettenkofer three years earlier; however “the true nature of these substances eluded him”, says Liebig). Liebig successfully marketed Liebig Fleish Extract (Extractum Carnis Liebigii), produced from leftover meat of cattle slaughtered for leather in Uruguay and Brazil. This product quickly became extremely popular in Europe, which was still plunged in the misery of war. First regarded as a remedy for the exhausted and wounded soldier on the field of battle, it soon found its way “into every grocer’s and chemist’s shop”. However, meat extract was also demolished because it consisted for most part of “inosine, creatine and creatinine and other excrementitious substances” with no nutritive value at all. “Heaven help the poor men taken into the field either to fight the battles of their country or to till its soil fed upon the homeopathic or infinitesimal diet described by Liebig”, raged the British chemist Thomas Vosper in a heated correspondence in the Lancet in which he addressed Liebig as “the learned Baron”, who in return questioned “Mr. Vosper’s competent knowledge on the subject.” In contrast, the British zoologist and Darwinist Ray Lankester, who was a great believer in the action of this meat extract “on exhausted nervous systems and deliberated frames”, suggested that the explanation of “the marvelous powers” should be sought in exactly the high creatine content, although little was known about its action on the human body. A certain professor Agassiz had recently proposed that creatine assisted in “nourishing the nerves” and recommended fish, known to contain the most creatine, as especially adapted for “the food of philosophers and those who work much with their brains”. Liebig Fleish Extract, which probably contained about 5% creatine, might be considered an early form of creatine supplementation. Von Liebig, who also introduced the first artificial infant food to the market (“Liebig’s Soluble Food for Babies”), died immensely rich and a baron in 1873.

Chanutin was, in 1926, the first to try true creatine supplementation (10 gram daily) on himself and his trusting assistant Loren Guy. He noted that a small amount of the ingested creatine was retained in the body which was followed by an increase in creatinine excretion in the urine. Both subjects gained 3 kg in weight.

Hunter devoted an entire monograph to creatine and creatinine. It provides a throughout overview of creatine contents of various organs of different species. It was

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* The creatine in urine was later found to be a secondary product, formed from creatinine due to procedures used.
estimated that skeletal muscles contain as much as 98% of total body creatine while three-fourths of the remainder are to be found in brain. The significance of creatine was still unknown. Although creatine had long been regarded as a waste product of protein catabolism, Hunter stressed that creatine must be “a substance with a function, a function in some way connected with (...) that capacity for rapid and powerful contraction which is the most important property of striated muscle”. And, he went on to state: “the presence of notable quantities in the brain would further indicate, that creatine is essential to the normal activity of (...) the entire neuro-muscular apparatus of voluntary movement”.14 Hunter’s book appeared in 1928 and he was just able to add an appendix on an observation, reported in 1927, which threw an entirely new light upon creatine and eventually revealed its importance in the energy metabolism of muscular contraction.

It must have been around Christmas 1926 that the British couple Philip and Grace Palmer Eggleton submitted their famous paper on a very labile form of organic phosphate, which they called “phosphagen” and which decreased during muscle fatigue.15 Fiske and Subbarow discovered this “labile phosphorus” at the same time and identified it as phosphocreatine.16 Phosphocreatine was the first compound found to carry the high energy phosphate bond and was at first considered the direct energy donor in muscle contraction.17 However, this key role was soon taken over by adenosine triphosphate (ATP) discovered by Karl Lohmann in 192917(and again simultaneously by Fiske and Subbarow, who were, much to their chagrin, too late with their publication this time).18 Lohmann and his colleague Lehmann also discovered the reversible conversion of creatine and ATP to phosphocreatine and ADP, catalyzed by creatine kinase (the famous “Lohmann” reaction). The role of phosphocreatine was thereafter reduced to a buffer for the resynthesis of ATP in muscle contraction.17 The creatine phosphate shuttle hypothesis, first proposed in 1972, showed that phosphocreatine is formed at the mitochondrial sites of energy production and moves to the cytosol where it resynthesizes ATP to allow continued muscle contraction. Herewith, phosphocreatine regained its key role in energy metabolism of the muscle.17

In the meantime, in the 30’s, Rudolf Schoenheimer introduced the isotope tracer technique to biochemistry, bringing about a rapid growth in the knowledge of biosynthesis.19 Thus it was also elucidated that arginine and glycine are converted to guanidoacetic acid (glycocyamine) which is then rapidly methylated to creatine with methionine as methyl group donor.20-22
Aims of this thesis

After Harris et al.\textsuperscript{23} showed in the 1990s that creatine supplementation increased the creatine content of skeletal muscle, creatine became a very popular supplement used by athletes to build muscle mass and improve performance.\textsuperscript{24} Because the brain may spend up to 20\% of the body’s energy consumption creatine was soon also considered as a therapeutic agent in many neurological conditions.\textsuperscript{25}

In 1994, the first patient with a complete creatine deficiency in the brain was reported. This was caused by deficiency of the guanidinoacetate-methyltransferase (GAMT) enzyme, catalyzing the second step of creatine biosynthesis.\textsuperscript{26} In 2001, Salomons and deGrauw discovered deficiency of the creatine transporter, responsible for the uptake of creatine into the cells, as another cause of cerebral creatine deficiency,\textsuperscript{27,28} and soon thereafter the first patients with cerebral creatine deficiency due to deficiency of arginine:glycine amidinotransferase (AGAT), catalyzing the first step of creatine biosynthesis, were described.\textsuperscript{29} Intellectual disability with behavioral abnormalities and seizures are the central clinical features in these syndromes. Therefore, the discovery of inborn errors of creatine synthesis and creatine transport now definitively draws the attention to the importance of creatine in the brain.

1.2 AIMS OF THIS THESIS

Since the discovery of the creatine transporter deficiency as a cause of X-linked intellectual disability, many questions regarding this new condition were to be answered. What is the phenotypic spectrum? How do we identify the patients? Is there a relation between genotype and phenotype? Because the molecular defect was discovered at the metabolic unit of the department of Clinical Chemistry of the VUmc in Amsterdam, this was the initial center worldwide performing DNA analysis of the SLC6A8 gene, receiving DNA samples from patients all over the world. We collected the clinical data of these patients and studied the phenotype and genotype in 101 male patients with X-linked creatine transporter deficiency (CRTR-D) (chapter 2.1).

Some patients have large SLC6A8 deletions extending beyond the 3’end of the gene. This appeared associated with a more severe phenotype. Around the same time contiguous gene deletions ABCD1 and BCAP31 (DXS1357E) just downstream of SLC6A8 were reported in patients with a severe phenotype named “contiguous ABCD1 DXS1357E syndrome” (CADSS). Is BCAP31 also involved in the severe phenotype of patients with SLC6A8 deletions? We characterized the deletions in eight patients with deletions of
SLC6A8, BCAP31, and/or ABCD1 and compared their phenotypes (chapter 2.2) to answer this question.

Patients with cerebral creatine deficiency due to defects in the creatine synthesis (AGAT and GAMT deficiency) profit from creatine supplementation. However, soon after the discovery of the CRTR-D it became obvious (and not unexpected) that creatine monotherapy was not effective in patients with CRTR-D. As brain cells are capable of endogenous creatine synthesis, supplementation with creatine precursors L-arginine and glycine appeared a good option to increase cerebral creatine synthesis. Treatment with creatine monohydrate, L-arginine, and glycine was started in the Erasmus MC in Rotterdam in nine Dutch boys with CRTR-D. We studied the effect of this treatment (chapter 2.3).

CRTR-D is an X-linked condition. Therefore X-inactivation plays an important role in the phenotype in female heterozygotes. This raises the following questions: Do females who are heterozygous for CRTR-D present with symptoms? How do we diagnose heterozygous females? What is the X-inactivation pattern in heterozygous females and is there a correlation with the phenotype? To answer these questions we studied the clinical features and X-inactivation patterns in eight Dutch females heterozygous for CRTR-D (chapter 3.1).

Not all the mothers of male patients with CRTR-D are carriers. The mutation frequently occurs de novo in an index patient. What is the recurrence risk of CRTR-D in further pregnancies of these mothers? Does germline and/or somatic mosaicism occur? We describe a mother of two affected sons in whom the mutation was not detected with sequencing of DNA from blood. Denaturing high-performance liquid chromatography (DHPLC) was used to detect somatic mosaicism (chapter 3.2).

Trying to understand the pathogenesis of CRTR-D, we are confronted with an important paradox: why does CRTR-D cause cerebral creatine deficiency while the brain cells synthesize their own creatine? This question is of paramount importance in the development of an effective treatment. We propose a new hypothesis about the pathophysiology of this disorder (chapter 4.1). An overview over the current clinical and pathophysiological insights regarding the creatine transporter and its deficiency, based on the results of this study and a review of literature, is given in chapter 4.2.
REFERENCES

8. Vosper T. Liebig’s extract of beef and food for infants. Lancet 1865;Oct 14:441-443
16. Fiske CH and Subbarow Y. The nature of the “inorganic phosphate” in voluntary muscles. Science 1927;65:401-403


