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Content of selected amino acids in the gastrocnemius muscle during experimental hypothyroidism in rats

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Abstract

Introduction: Thyroid hormones affect protein turnover, and in the case of hypothyroidism a decrease in protein synthesis and reduced release of certain amino acids from skeletal muscles are observed. Changes in the amino acid system of skeletal muscles may be responsible for the occurrence of muscle disorders. **Material and Methods:** The study measured the content of selected amino acids in the gastrocnemius muscle of Wistar rats during experimental hypothyroidism induced by oral administration of methimazole at a concentration of 0.05% in drinking water for 90 d. The rats were divided into four groups: E1 (n = 6) – experimental males, E2 (n = 6) – experimental females, C1 (n = 6) – control males, and C2 (n = 6) control females. **Results:** A statistically significant reduction occurred in leucine, isoleucine, and 1-methylhistidine levels in males, and 1-methylhistidine in females, in comparison to the control groups. **Conclusion:** The hypothyroidism-induced changes in amino acid content may be responsible for the occurrence of skeletal muscle function disorders.

Keywords: rats, hypothyroidism, amino acids, muscle.

Introduction

Skeletal muscles play an important role in amino acid metabolism and represent the body's largest protein reservoir. The branched-chain amino acids are the most effective precursors for alanine formation in muscles. In this regard, they play an important role in glucose homeostasis by determining the availability of alanine as a gluconeogenic precursor (24). Muscle amino acid metabolism is altered during stress, trauma, burn, sepsis, obesity, acute uraemia, and in the course of other diseases (22). The content of amino acids in muscles influences protein metabolism – for example branched-chain amino acids such as leucine primarily stimulate muscle protein synthesis in healthy rats (14).

The thyroid hormones (THs: 3,5,3′,5′-L-tetraiodothyronine (thyroxine, T_4) and 3,5,3′-L-triiodothyronine (T_3)) stimulate protein turnover. Hypothyroidism leads to a decrease in protein synthesis and to the release of such amino acids as alanine, tyrosine, glycine, and glutamine from the skeletal muscles. In the case of hyperthyroidism, muscle protein synthesis decreases, while amino acid release from the muscles due to proteolysis increases (2, 17, 18, 23). THs have a major effect on muscle formation. if the thyroid is removed after birth, the muscles of rats are pale, weak, and have reduced volume (31). During hypothyroidism, clinical disorders of their contractile properties are observed as well as myopathies, such as myasthenia and myotonic dystrophy (19, 21, 31).

The above data indicates that disorders of the amino acid composition of skeletal muscles may occur during hypothyroidism. Determination of amino acid content in skeletal muscles may enable better understanding of functional muscle disorders during hypothyroidism. The aim of this study was to assay variability and changes in selected amino acid contents in muscle during experimental hypothyroidism.

Material and Methods

Animals. Wistar rats of both genders (220–260 g) were divided into four groups: C1 (control group) – healthy males (n = 6); C2 (control group) – healthy females (n = 6); E1 (experimental group) – males receiving methimazole (n = 6); and E2 (experimental group) – females receiving methimazole (n = 6). The rats were kept in an air-conditioned room with average humidity of 45%–47%, temperature of 22–23°C and 12/12 light cycle. The rats underwent 14 d adaptation prior to the experiment. After this period, they were fed a commercial diet for laboratory animals (Agropol, Poland). Rats from control groups had access to tap water *ad libitum*, but the rats from experimental groups were given 0.05% methimazole (Sigma-Aldrich, USA) water solution, administered *ad libitum*. Fresh solution was prepared daily.

Analysis of amino acid concentrations in the gastrocnemius muscle. After 90 d, all animals were anaesthetised using ketamine (80 mg/kg body weight i.m.) and gastrocnemius muscle samples were obtained immediately after euthanasia and frozen at −25°C. The whole gastrocnemius muscle from each rat was weighed and homogenised in 10 mL of 6.0% sulphosalicylic acid buffered to pH 2.9. The homogenised 1 g samples were centrifuged for 15 min at 12,000 g. The obtained supernatants were used for free amino acid determination with the use of ion-exchange chromatography and an INGOS AAA-400 apparatus for automatic analysis of amino acids (Ingos Corp., Czech Republic). Amino acids were separated using an OSTION LG FA 3 mm × 200 mm analytic column (Ingos Corp., Czech Republic). For separation of the acids five lithium citrate buffers (pH 2.9, 3.1, 3.35, 4.05, and 4.9) were used. The separated amino acids were subjected to ninhydrin reaction and their identification was performed on the basis of retention time in comparison to the standard using a photocell combined with a computer. MIKRO software, version 1.8.0, was used for amino acid evaluation (Ingos Corp., Czech Republic).

Statistical analysis. All values are presented as means ±SEM. Statistical analysis was performed using Statistica software, version 10.0, and a non-paired Student's *t*-test related to each sex. The differences between mean values were considered as statistically significant at $P < 0.05$.

Results

A The results related to the content of selected amino acids in the gastrocnemius muscle are presented in Table 1.

Table 1. Content of amino acids in the gastrocnemius in μmol/g of tissue

Group		**ADI**	**ALA**	**ARG**	**ASP**	**CYSTA**	**ETA**	**GABA**	**GLN**
C1	x	85.67	3892	564.7	399	15.5	255	28.67	1137
	SEM	26.15	151.63	2.87	10.74	5.51	0.33	9.04	177.15
C2	x	102.8	4196	633.3	464.3	24.67	263.8	32.75	1671
	SEM	33.38	175.16	121.0	44.08	12.8	28.78	11.16	225.27
E1	x	192.8	3455	522	380	32	241.3	14.83	1464
	SEM	41.39	269.97	27.0	33.71	14.26	8.64	6.87	102.47
E2	x	92.17	3746	667.5	465.2	11.67	234.2	63.33	2057
	SEM	28.34	461.51	72.52	49.26	4.72	9.94	32.05	257.2
		GLU	**GLY**	**HIS**	**ILE**	**KCYS**	**LEU**	**LYS**	**MET**
C1	x	2973	4497	306	311.7	143.7	582	1098	157.3
	SEM	199.41	254.01	14.49	8.19	17.93	21	45.5	7.81
C2	x	3668	3780	337.3	326	116.5	637.3	1273	186.8
	SEM	342.67	471.36	42.33	85.18	7.77	156.53	220.79	61.8
E1	x	3560	3991	294.7	251.2*****	121.5	458.7*****	833.3	150
	SEM	377.36	274.71	11.17	14.28	10.08	21.01	101.01	10.54
E2	x	3753	4457	335.3	310.3	125.2	616	1133	183.8
	SEM	473.76	325.43	29.88	45.4	12.89	94.0	221.17	32.16
		ORN	**PHE**	**SER**	**TAU**	**THR**	**TYR**	**VAL**	**1MH**
C1	x	39	304	1380	14034	1245	283.7	521	2237
	SEM	3.78	10.93	41.65	1037.59	74.24	14.38	19.74	63.33
C2	x	78	343.3	1524	9071	1366	326	570.3	1876
	SEM	33.05	87.29	150.96	1887.83	182.85	90.42	125.52	81.3
E1	x	37.8	251.8	1522	10405	1084	218.2	429.3	1397*****
	SEM	2.81	6.98	102.34	2355.49	65.42	12.03	13.88	129.87
E2	x	68	338.2	1391	10017	1400	311.8	531.2	1402*****
	SEM	2.08	44.69	297.01	2339.36	110.28	37.08	74.63	144.83

C1 – control group – males, C2 – control group – females, E1 – experimental group – males, E2 – experimental group – females. ADI – aminoadipic acid, ALA – alanine, ARG – arginine, ASP – aspartic acid, CYSTA – cystathionine, ETA – ethanolamine, GABA – γ-aminobutyric acid, GLN – glutamine, GLU – glutamic acid, GLY – glycine, HIS – histidine, ILE – isoleucine, KCYS – cysteic acid, LEU – leucine, LYS – lysine, MET – methionine, ORN – ornithine, PHE – phenylalanine, SER – serine, TAU – taurine, THR – threonine, TYR – tyrosine, VAL – valine, 1MH – 1-methylhistidine. * – statistically significant differences compared to control group (E1–C1, E2–C2)

Compared to group C1, in group E1 there was observed a reduction of arginine, cysteic acid, taurine, aspartic acid, threonine, glycine, alanine, leucine, valine, 1-methylhistidine, methionine, isoleucine, tyrosine, phenylalanine, γ-aminobutyric acid, ethanolamine, ornithine, lysine, and histidine, with a simultaneous increase in serine, glutamic acid, glutamine, aminoadipic acid, and cystathionine contents. However, statistically significant differences only marked out the leucine, isoleucine, and 1-methylhistidine contents. Different results were obtained for group E2 in comparison to group C2, where the amino acid analysis showed a reduction in the contents of serine, aminoadipic acid, alanine, cystathionine, valine, 1-methylhistidine, methionine, isoleucine, tyrosine, phenylalanine, ethanolamine, ornithine, lysine, and histidine, with increased contents of cysteic acid, taurine, aspartic acid, threonine, glutamic acid, glutamine, glycine, γ-aminobutyric acid, and arginine. However, a statistically significant difference was only seen for 1-methylhistidine.

Discussion

The muscular disorders observed in hypothyroidism are caused by numerous factors and they have not been explained completely to date, although there are several principal elements that have a direct effect on skeletal muscle growth and protein turnover. For example, rats subjected to the experimental model of methimazole-induced hypothyroidism exhibit a reduction in myostatin (MSTN, previously known as growth differentiation factor-8) expression, which is the strongest skeletal muscle growth inhibitor. This key protein prevents proliferation and differentiation of myoblasts and slows protein synthesis in cells (28, 31). By contrast, THs-dependent insulin-like growth factor I (IGF I) and insulin-like growth factor II (IGF II), which are in opposition to myostatin, constitute a positive factor for the differentiation, growth, and regeneration of muscles (12, 15). Another element regulating the effect of THs on muscle tissue condition and metabolism, including protein turnover and stability of the cytoskeleton and intercellular connections, is expression of the thyroid receptor (TR, part of the nuclear receptor superfamily). TR induces many genes important for this phenomenon: most of them participate in the transcriptional and post-transcriptional regulation of protein synthesis (2, 4).

Thyroid hormones are regulators of development, differentiation, repair, and metabolism, and their primary function is regulating basal metabolic rate (16). T_3 affects skeletal muscle development by stimulating the increase in diameter and number of muscle fibres (26). It also induces the expression of myoblast protein 1, which plays an important role in activating satellite cells, enabling differentiation and proliferation of new muscle cells during tissue repair (16). A deficit of THs leads to a reduction of muscle fibre efficiency, manifesting in weakening and fatigability of muscles, as well as pain and contractures (21, 29). Muscle tissue contains almost half of the organism's proteins, and forms the most important reservoir of amino acids, while protein turnover and the fate of muscle amino acids are regulated by THs (30, 31).

In the experimental model of hypothyroidism, we have achieved both an increase and decrease in the content of individual amino acids in the gastrocnemius muscle. Whether it was an increase or a decrease typically depended on the sex of the tested rats. In most cases, deviations from normal values (control groups) were minor, and statistically significant differences in comparison to the control groups appeared only for leucine (decrease in males), isoleucine (decrease in males), and 1-methylhistidine (decrease in males and females). a decrease in the content of these extremely important amino acids potentially has serious repercussions for the functioning of the skeletal muscles. Disorders in thyroid functioning lead to changes in leucine metabolism (17). Leucine is an essential amino acid with a branching structure and a twin nature: it exhibits both anabolic and catabolic activities. It stimulates oxidative metabolism, especially that of 5′adenosine monophosphate-activated protein kinase (AMPK), which is the main regulator of cellular energy management (27). In the case of leucine supplementation in Ay mice, increased oxygen consumption was observed, suggesting increased energy expenditure through selective stimulation in the expression of genes important in the metabolism of fatty acids and in the biogenesis of mitochondria in the skeletal muscles (10). It is also notable as a stimulator of muscular metabolism, boosting cellular synthesis of proteins and participating in mRNA translation. *In vitro* tests performed on mouse C2C12 cellular lines demonstrated that subjecting them to leucine starvation lowers protein synthesis in myoblasts (27). This amino acid also plays an important role as a repair factor. When supplemented to rats with muscle damage, it reduces the size of inflammation areas in the muscles, causes an increase in the number of proliferating muscle fibres, and thus contributes to regeneration of muscle fibres, modulating components of the phosphoinositide 3-kinase/Akt-protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway and ubiquitin-proteasome system (20). Isoleucine is an isomer of leucine and similarly must be provided to the organism in food (11). However, it plays a slightly different role; in particular it is a factor in carbohydrate metabolism. It stimulates the capture and, independently from insulin and mTOR, transport of glucose in skeletal muscles (5). In contrast to leucine, it does not exhibit anabolic properties by increasing protein synthesis in skeletal muscles: it does

not increase the activation of translation initiation factors (7). An entirely different role in skeletal muscles than that of branched amino acids belongs to 1-methylhistidine. This amino acid is part of the dipeptide anserine, which exhibits anti-oxidation and anti-inflammatory activity (3, 25). Importantly, anserine removes and directly decomposes one of the most common and cytostatic lipid-derived reactive carbonyl species, 4-hydroxy-trans-2-nonenal (HNE) (25). Moreover, 1-methylhistidine is an object of interest as a urine and serum biomarker of drug-induced skeletal muscle toxicity and hypertrophy (1).

Muscle fibres exhibit variation depending on the type of proteins they contain. The isomorphs of myosin characterised so far differ in amino acid composition, and the proportions of different isoforms decide the functional characteristics of the muscles, despite similar biological activities. The activity of glycolytic enzymes in muscles is closely dependent on the type of muscle fibres. The ratio of oxidative to glycolytic enzymes represents the metabolic properties of muscles and oxygen-dependent metabolism of glucose and fatty acids (6).

Disorders resulting from methimazole-induced hypothyroidism result from the drug's effect and hypothyroidism *per se*. This is particularly important in patients with hyperthyroidism inadequately monitored during the treatment. In some studies, all monitored patients had hypothyroidism after 12 weeks of methimazole treatment at a usual dose of 40 mg/day (13). For this reason, the experimental model used in the study has great clinical importance. In conclusion, protein turnover disorders occurring as a result of methimazole-induced hypothyroidism lead to changes in the content of many amino acids in muscles. In particular, a lowered content of leucine, isoleucine, and 1-methylhistidine may engender serious consequences for the functioning of skeletal muscles, including reduction in protein production, weakened regeneration of muscle fibres, degradation of glucose capture and transport in males, and muscle exposure to the results of oxidative stress in both males and females.

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