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β-Hydroxy β-methylbutyrate (HMB) supplementation during pregnancy and perinatal period in animals studies and possible application in humans

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Abstract

β-Hydroxy β-methylbutyrate (HMB) is a diet supplement known for its positive impact on muscle anabolism, which makes it very popular among amateur sportsmen. Diet enriched with HMB leads to decreased proteolysis in muscle tissue, which is extremely important in the process of muscle regeneration after intensive training. As the number of possible implications of this molecule in medicine is still growing, the aim of this review was to find potential applications of HMB in women and their infants during pregnancy and perinatal period.

The HMB usage during pregnancy or shortly after birth is connected with increased muscle anabolism in the infants. Many factors like mTOR signaling pathway, increased activity of GH/IGF-1 axis or elevated blood concentration of some amino acids may be responsible for this effect. Such activity might be potentially helpful for infants born with intrauterine growth retardation (IUGR). Improvement in quality and quantity of colostrum and milk, positive impact on bones and cartilages or immunostimulating effect and deceased enamel roughness, which potentially reduces the risk of caries, are other potential benefits. Although numerous possible positive effects have been postulated, there seems to be insufficient data on safety of long term HMB supplementation, especially in pregnancy and perinatal period. Moreover, there are studies that imply increased peripheral insulin resistance after prolonged supplementation of HMB.

HMB usage can be beneficial in numerous health states, also in pregnancy and perinatal period, especially in infants with low birth weight or born preterm, but further investigation is needed to estimate benefits to risk ratio and introduce specific guidelines.

Introduction

β-Hydroxy β-methylbutyrate (HMB) is one of the leucine metabolites naturally appearing in the organism. It is produced from small amount of all α-ketoisocaproate in the reaction catalysed by α-ketoisocaproate dehydrogenase (1). Since Nissen and his colleagues took note of it in 1992, HMB has been considered to be used in many applications. For now usage among sportspeople as an addition to appropriate training is the most popular one, as the supplement helps to increase muscle mass, regenerate and decrease proteolysis in skeletal muscles or reduce the amount of fatty tissue (2). There are more and more studies which refer to possible medical applications of HMB or its combinations with other nutrients. Experiments conducted on animals as well as on humans deal with treatment of diabetic foot ulcers (3), muscle atrophy in elderly patients or in those with cancer or AIDS (4). There is also a study conducted on rodents, which suggests, that HMB supplementation reduces tumour growth by modification of transcription factors like NF-κB (5). Another study conducted on calves, in which researchers noticed increased γ-globulins and lysozyme concentration, gave a basis for further analyses of HMB influence on immune system (6).

This article aims to review consequences of HMB usage in pregnant females and their offspring during perinatal period in animals studies. It also suggest potential usage in human infants and outline connected research problems.

Despite the fact that HMB is widely available, there's no certainty about its safety. It was proven that 4 week supplementation with daily dosage 320 mg/kg of rat body mass caused increased peripheral insulin resistance (7, 8), which casts doubt on the supplement as it might be connected with type II diabetes. In another study average dose of 3493 mg/kg/day for male and 4163 mg/kg/day for female was administered and no anomalies in the results of laboratory tests and in any internal organs were noted, but the glucose tolerance test or insulin level measurement haven't been done (9). The issue of HMB usage safety needs to be settled in further investigation, especially when we think about possible development of insulin resistance and type II diabetes.

Supplementation during pregnancy

Even if we think about HMB supplementation during pregnancy, still the influence on newborns is the main research problem that appears in the reviewed studies. However, in one study researchers focused on the effects on females. Pregnancy and lactation period is challenging for mother's organism as its metabolism is focused on processes, which ensure proper development of the fetus and optimal milk composition. Tomaszewska *et al.* in their experiment conducted on female minks stated that daily HMB at the dose of 0.02 g/kg of mink body mass during pregnancy improves long bones mechanical parameters measured after parturition (10). If we would like to shift these results to human population, they could seem irrelevant as mothers' bones return to their original mass in 6-12 months after the end of lactation (11). However, there are some particular cases of increased fracture risk during pregnancy caused by calcium metabolism disorders, in which HMB application could be helpful. It also appeared that pregnancies increase fracture risk in women, who used oral contraception – especially in 4 and more pregnancies and when contraceptives had been taken after the age of 30 (12), so it might be another field of potential HMB usage.

Among published studies on HMB effects in pregnancy predominate ones conducted on pigs. They differed in the substance dose, pregnancy stage and duration of administration. These variations do not help in drawing unambiguous conclusion or summing up the topic briefly, and that's why findings of particular experiments will be presented below. But firstly it's worth to notice the following spiny mice study, as it is the only one of this kind in our review. In this interesting study tooth enamel parameters in newborn spiny mice were observed. In the experiment Świetlicka *et al.* showed, that 0.2 and 0.02 g/kg of body mass HMB supplementation in pregnant mothers in the last stage of pregnancy decreased incisors enamel roughness in their offspring. This effect was greater in males than in females but both were statistically significant. Decreased enamel roughness restricts markedly bacteria adhesion on its surface and reduces caries risk at once (13).

Wan *et al.* administered 4 g of HMB daily to pregnant sows starting from 35th day of pregnancy and continuing to the parturition. At first, researchers noticed that in the experimental group the number of stillborn piglets was lower than in the control group, which may be a result of increased IGF-1 (insulin-like growth factor-1) concentration, and thereby stimulation of fetus development and progesterone production in the early stage of pregnancy. In addition, birth weight of piglets from supplemented sows was higher and also in this group the number of low birth weight piglets was lower, which was ascribed to intensive development of skeletal muscle in prenatal period, and this fact was confirmed by further observations. Longissimus dorsi and semitendinosus muscles mass was higher in piglets from the experimental group than in the controls. Likewise, mRNA levels of myogenic genes: MRF4 (muscle regulatory factor 4), MyoD (myogenic differentiation factor); and IGF-1 in these muscles were higher. Also creatine kinase concentration was increased, which suggests intensified muscle metabolism. What's interesting, decrease in food intake was observed during lactation period in sows supplemented with HMB while being pregnant; authors of the study connected this fact with hyperinsulinemia that appeared in the final stage of pregnancy; and hyperinsulinemia in connection with increased glucose concentration (14) is in the accordance with mentioned earlier insulin resistance development caused by HMB administration. Sows from experimental group had also improved colostrum quality (higher content of protein and lactose) and increased quantity of fat in the milk (14).

Tatara *et al.* in their study, in which HMB was administered to sows in the last 2 weeks of pregnancy, noticed that β-hydroxy β-methylbutyrate increased both piglets birth weight and daily weight gain, but final body mass (after 90 days) wasn't higher than in the piglets from the control group. In the piglets from the HMB group serum levels of glutamine, valine, tyrosine were increased, and according to the researchers this might be one of the reasons for faster piglets growth. Another stimulating factor for piglets development may be increased activity of GH/IGF-1 axis. Just after birth GH (growth hormone) level in the offspring of mothers fed with addition of HMB was increased, but after 90 days returned to the normal value. Something different was observed regarding IGF-1 concentration, which was increased equally right after birth and after 90 days. The fact that HMB had anabolic effect on bone tissue was new observation – BAP (bone-specific alkaline phosphatase) level was decreased both at the beginning and at the end of observation; likewise, femur mass was augmented in comparison with the control group – most likely as a result of the improvement of bone geometry and its volumetric mineral density. Results above are also ascribed to increased activity of GH/IGF-1 axis, but it is probable that higher muscle mass contributed to mechanical bone parameters improvement (15).

In the experiment conducted by Flummer *et al.*, which aimed to determine the influence of HMB supplementation during the late stage of sows' pregnancy (10 days before parturition) on body composition of the offspring, it was stated that in the carcass obtained from 28-day-old piglets there was an increased content of crude protein whilst the fat content was decreased. Piglets' internal organs, like liver, spleen, kidney or caecum, were heavier. At the same time a decrease in in the mass of the small intestine was observed. There was no effect on piglets' birth weight, daily weight gain, weight at the age of 28 days or heart, stomach or the rest of large intestine mass. Researchers point out that obtained results might indicate the shift in piglets metabolism to protein synthesis and lipolysis. According to the authors, higher liver and spleen mass might be connected with increased liver metabolic capacity and improved immune system function respectively, or intensified HMB metabolism in these internal organs (16).

Blicharski *et al.* have supplemented pregnant sows with HMB at a daily dose of 0.2 g from 70th to 90th day of gestation (17). Piglets organs were removed right after birth, and results of the measurements only partially agreed with results obtained by others researchers. Particularly liver, stomach, lungs and brain mass in males were increased, and these effects were not so apparent in females; however, spleen weight wasn't higher. Lower blood level of calcium and phosphorus was noticed, which may suggest intensified bone uptake of these elements, and this effect might be confirmed by following observations on piglets skeletons. The length and diameter of femur were increased; also mechanical and geometrical bone parameters improved, but again, the change was more substantial in males than in females. Likewise, cartilages mechanical properties developed; a rise in the content of proteoglycans was noticed. It is known, that they decide about cartilage characteristics together with collagen. Like in other studies, Blicharski observed increased synthesis of collagen due to HMB supplementation. Authors correlated the expanded newborns development to the elevated IGF-1, but also to leptin level. What is interesting, increased activity of pituitary-gonadal axis was noticed, which revealed itself in higher FSH (follicle-stimulating hormone), LH (luteinizing hormone), estradiol and testosterone concentrations in experimental groups of females and males (17).

Another study exceeding pregnancy period was published by Flummer *et al.* They administered HMB at a daily dose of 2.5 g to sows equally during the last week of pregnancy and the first 28 days after the parturition during lactation period. It was shown that sows from the experimental group produced more colostrum with no difference in its composition in comparison to the control group. Furthermore, the milk from sows in the experimental group had higher fat content, which agrees with previously cited articles. No differences were observed in birth weight of the newborns between the study and the control groups (18), which is unequivocal with the other researchers observations. The cause of these discrepancies might be shorter time from the beginning of the supplementation to the parturition, which in this case was only one week. Another reason may be low HMB dose used in the experiment. What is interesting, reduced sows backfat was observed at weaning, which suggested possible lipolytic properties of β-hydroxy β-methylbutyrate (18).

Supplementation during lactation

It was proved in another study conducted on pigs, that the enrichment of the lactating sows diets with HMB had substantial influence on selected parameters in the sucklings. HMB was administered during lactation and piglets were observed between 28th (day of weaning and slaughter of the part of the piglets) and 180th day of life. While investigating HMB transfer to the milk and its influence on skeletal muscle of the offspring, Wan *et al.* noticed that, in spite of the same food intake among piglets, these from supplemented mothers gained higher final mass and daily average weight gain. There was an increased percentage of lean meat in the carcass obtained from the experimental group piglets, which practically suggests higher muscle tissue content in the carcass. At the age of 28 days the blood concentration of insulin and glucose in the piglets from the experimental group were augmented in comparison to the control group, which agrees with the mentioned above possible risk of insulin resistance caused by the dietary intake of β-hydroxy β-methylbutyrate. While observing the influence of the tested diet on skeletal muscle researchers stated, that – in comparison to the control group – the cross-sectional area of type II muscle fibres was expanded (19). Both increase in mentioned cross-sectional area and higher level of mRNA of myosin heavy chain isoform IIb (MyHC-IIb) in longissimus dorsi muscle the day 28 and the final day of the experiment may suggest muscle fibre conversion from slow-twitch to fast-twitch fibre (19), as MyHC-IIb is a marker of muscle fibre type – in this case fast-twitch IIb type (glycolytic) fibre (20). Overall boosted muscle development in piglets is elucidated by following authors findings: increased level of mRNA of mTOR and transcription factor Sox6 was found in longissimus dorsi of piglets from the experimental group at weaning, and higher blood concentration of leucine, which stimulates muscle protein synthesis was noted. No integroup difference was observed in the content of mRNA of MAFbx and MuRF1, which are muscle protein degradation markers, and according to the authors this fact indicates lack of HMB influence on muscle proteolysis. It was also confirmed, that supplemented sows ate less (19).

Supplementation in newborns

An interesting study was conducted on piglets with intrauterine growth restriction (IUGR); 800 mg HMB/kg of the piglet body mass was administered between 7 and 28 day after birth. It turned out that IUGR piglets fed with HMB diet attained similar weight and daily weight gain to those, which were not supplemented. This is in agreement with other researchers' observations: the increased mRNA amount of mTOR, MyHC-IIb and IGF-1 in the longissimus dorsi muscle, and the enhanced cross-sectional area of type II muscle fibre, which may be connected with higher lactate dehydrogenase concentration in the muscle. Furthermore, mTOR level was higher in IUGR piglets than in those from the control group, which even more trenchantly shows HMB effectiveness in equalizing parameters in newborns with low birth weight. In both experimental groups increased creatine kinase levels were observed. It may indicate an elevated amount of glycolytic fibres and overall intensified muscle development (21).

Another experiment was conducted on piglets in the second day after birth – HMB was administered directly into the carotid artery and the internal jugular vein: during first 10 minutes in doses 0, 12, 60 and 240 μmol per kg of body mass per hour, and for further 50 minutes in doses 0, 20, 100 or 400 μmol per kg of body mass per hour, respectively. It was stated that in HMB 20 group protein synthesis intensity in longissimus dorsi, diaphragm, gastrocnemius and soleus muscle, as well as in lungs and spleen was higher, which was caused by mTOR signalling pathway activation. But it was a different matter in HMB 100 and 400 groups, where increased protein synthesis was observed only in longissimus dorsi or wasn't observed at all (22), which might suggest that exceeding the supplement dose not only doesn't increase its influence on protein synthesis, but also may decrease the influence. The blood analysis revealed that in the two groups which received the highest doses of HMB there was considerably increased glucose level, but insulin level did not differ between the groups (22). Perhaps the short time of HMB exposition does not cause insulin resistance, but at the same time it improves protein synthesis parameters in muscles. Interesting observation was the fact that HMB activity seems to be selective to skeletal muscles – there was no difference noticed in the intensity of protein synthesis in any internal organs apart from lugs and spleen. No HMB influence was noticed on muscle protein degradation or amino acids transporters (LAT1 and SNAT2) in the muscles (22).

An interesting perspective on HMB supplementation is provided by birds studies though it is difficult to refer them to mammals, and because of that only two of them will be described here shortly. Turkish study showed that HMB solution injection into broilers eggs the day 18 of incubation increased intestinal villi length and stimulated intestinal flora development, and thanks to that just 4 days after hatching chickens were more resistant to infections. Antibodies level was also measured and it was higher in the experimental groups than in the controls. The best effects both in lengthening intestinal villi and increasing antibodies level were achieved by *in ovo* injection of 0.2% HMB solution (23). Tako *et al.* in their study noticed that HMB *in ovo* injection increased villi surface area and disaccharidases activity in hatching birds, which influenced animals body mass at the end of their experiment (24).

Summary

HMB positively influences muscle development in newborns, which at the same time improves their body mass. The same as leucine, it's metabolite activates mTOR signaling pathway, which stimulates protein synthesis – also in the muscles (25). HMB increases GH/IGF-1 axis activity and amino acids blood concentration, and these factors are interdependent in the process of organism growth. But the fly in the ointment is the fact that these factors may also cause the insulin resistance (26). HMB supplementation may decrease tissue insulin sensitivity. Perhaps this adverse effect might be eliminated by dosage and exposure time adjustment. Because it stimulates newborns growth, HMB might be potentially used in infants with low birth weight in order to increase their body mass. However, the fact of growth restriction in prenatal development predisposes to obesity and diabetes later in life (27). There are some studies suggesting that fast body mass gain in infants increases the risk of obesity or insulin resistance in adulthood independently of their birth weight, but in case of preterm born babies it might be beneficial in terms of nervous system development (28). HMB influences colostrum and milk quality and may also have a positive impact on infants growth.

Positive impact of HMB on bones and cartilages, suggested (but not confirmed in all studies) lipolytic activity or especially interesting influence on immune system. This will need further investigation in order to prove those features, but also to evaluate perspectives of usage in humans. Although existing studies shaded some light on possible HMB applications, further research problems emerge: how HMB acts on the fetus when administered during pregnancy – is this direct action caused by crossing the placenta or rather arises from improvement in some parameters in mother's organism or better placenta development.

It seems that HMB usage during pregnancy or perinatal period may be beneficial for both: mother and offspring. However, this issue needs further exploration so that effectiveness, potential risk and long-term effects of supplementation in such a critical period of life could be thoroughly evaluated.

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