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What is the Actual Molecular Weight of Irisin Hormone According to Western Blot Analysis?

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Irisin hormone, secreted mainly in skeletal, cardiac muscles, is proteolytically cleaved from the C-terminal moiety and secreted from the fibronectin domain-containing protein 5(FNDC5) receptor. This hormone carries carbohydrate moieties, which are glycosylated, and is a dimeric protein, and released as a hormone of 112 amino acids [1]. The dimerization of this hormone is not affected by glycosylation, although N-glycosylation is necessary for the stabilization of FNDC5 and secretion of irisin [2]. Quantitation of circulating human irisin by Tandem Mass Spectrometry was ~ 3.6 ng/ml in sedentary individuals [3]. Irisin is secreted mainly in skeletal, cardiac muscles and adipose tissues.

Irisin immunoreactivity has also been documented in nerve sheath, salivary glands, ovaries, testes, rectum, pancreas, intracranial arteries, tongue, optic nerve, stomach, neuronal cells, and sebaceous glands. One of the most important functions of irisin is the change of subcutaneous and visceral adipose tissue into brown adipose tissue, and regulates glucose homeostasis and thermogenesis of body [4]. However, the validation of the western blot size of Irisin has been still debated. The Abcam polyclonal irisin antibody in western blot analyses was used in the first study [1].

This antibody is described in the Abcam catalog (ab117436) as being made against a peptide corresponding to C-terminal aa 149-178 of the human FNDC5 (aa 146-175 in mouse) [1]. This first, study has shown that irisin gives multiple bands, such as 32 kDa and 20 kDa, in western blot analyses [1]. It has been demonstrated with the PNGase F enzyme procedure (which separates sugar moieties from the protein) that these multiple bands are caused by sugar moieties bound to irisin [1]. This irisin cleaved from the sugar moieties has a lower molecular weight. More recently, Dr Sahna and his coworkers reported that the molecular weight of irisin is 90 kDa, which is theoretically 7.5 times higher than that of water-soluble irisin and 2.8-4.5 times higher than that reported previously on the basis of western blot analyses [1,4,5].

However, the Abcam monoclonal irisin antibody (rabbit monoclonal antibody against irisin (FNDC5 cat no: ab174833; Abcam) used by Dr. Sahna et al. is different from the Abcam polyclonal irisin antibody [5]. Recently, Roca-Rivada et al. Compared Abcam and Phoenix Pharmaceutical antibodies and showed that irisin bands formed could be between 10 and 60 kDa [6]. However, in this study, plasma was not investigated and only muscle and fat tissues were investigated after exercise.

Both the Abcam and Phoenix Pharmaceutical antibodies formed sharp bands of 25 kDa. Additionally, the Abcam antibody formed a faint band of 50 kDa [6]. However, compared with the previously reported data on the molecular weight of irisin, the 90-kDa molecular weight of irisin reported by Dr. Sahna and his coworkers on the basis of western blot analysis appears to be impossible. We warned them via editor of journal Journal of pineal research [5]. (They might possibly publish an erratum at this matter).

The protein reported in their study might be another protein with a molecular weight of 90 kDa that might be a cross-reacted with irisin [5]. Based on all the aforementioned information, it can be suggested that validation has not yet been conducted fully for commercial antibodies that are claimed to perform irisin analyses. Currently, the molecular weight of irisin has been generally reported as 10, 20, 24, 25, and 32 kDa based on western blot analysis study [1,4,6]. Therefore, according to the previously available data we believe that it would be beneficial to carefully reinterpret the molecular weight of irisin that has been reported as 90 kDa by Dr. Sahna and his coworkers [5].

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