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Research Article

CHANGES IN SECONDARY STRUCTURE OF PROTEIN IN SKELETAL MUSCLE DUE TO HIGH-CARBOHYDRATE OR HIGH-FAT DIETS

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ABSTRACT

Objective: Obesity, which arises from changes in lifestyle and feeding habits, poses a threat to human health. One essential contributor to the increase in obesity rates is the popularity of high-calorie diets. This study aims to investigate high-fat (HFD) and high-carbohydrate (HCD) diet-induced molecular changes in protein secondary structure in longissimus dorsi skeletal muscle tissues of female inbred C57BL/6J mice by utilizing Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy.

Materials and Methods: Mice were fed a control diet, HCD, or HFD for 24 weeks. Their skeletal muscle tissues were collected, and their spectra were recorded using a Bruker Invenio S ATR-FTIR spectrometer in the 4000-400 cm^{-1} region.

Results: The protein secondary structure profiles of the HCD group demonstrated a significant rise in antiparallel β-sheet and β-turn and a decline in parallel β-sheets, together with the insignificant increase in aggregated β-sheets and a decrease in α-helix. The impact of an HFD on protein conformation is less pronounced than HCD. The HFD diet led to an increase in antiparallel β-sheets and a decrease in parallel β-sheets. Although insignificant, an increase was observed in β-turn and α-helix.

Conclusion: These results propose the appearance of protein aggregation and/or formation of protein-protein intermolecular interaction in skeletal muscle tissues of female inbred C57BL/6J mice. Collectively, these data suggest that both high-calorie diets impair secondary structures of protein in skeletal muscle that may affect its metabolic function.

Keywords: Obesity, High-carbohydrate diet, High-fat diet, Protein secondary structure, FTIR, Spectroscopy

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INTRODUCTION

High-calorie diet consumption results in metabolic complications involving obesity, insulin resistance, type-2 diabetes, and metabolic syndrome that are associated with disruptions in lipid, protein, and carbohydrate metabolism (1). Skeletal muscles are profoundly involved in the modulation of this metabolism and undergo a range of structural and functional alterations in response to impaired insulin function due to ectopic lipid infiltration. This lipid accumulation in skeletal muscles and their intracellular localization is linked to alterations in tissue composition, structure, and remodeling (2). Changes in skeletal muscle macromolecular composition associated with obesity may thus provide insight into the mechanisms behind insulin-related diseases (3). Skeletal muscle not only aids in physical activity by producing mechanical work, but it also helps maintain health by utilizing and storing macronutrients. It uses a large proportion of the amino acids discharged into the circulating system from diets to construct novel functional proteins and is thus the key determinant of glucose and lipid uptake. Furthermore, alterations in the contribution of skeletal muscle to basal and/or subsequent macronutrient metabolism may have a substantial impact on disease threat. Myofibrillar proteins are especially vulnerable to anabolic resistance, and emerging evidence suggests that obesity can also unfavorably influence muscle protein turnover or the breakdown and restoration of functional proteins (3-5). Changes in protein structures and thus functions do not depend solely on modifications to the expression of genes or the production of proteins. To comprehend the functional abnormalities that manifest in various disease states and create new therapeutic strategies, it is crucial to ascertain the structural alterations of proteins and lipids (6). The ability of proteins to perform their duties fully depends on their three-dimensional structure in their natural state (7). Any mistakes that could happen during the folding step cause proteins to reach various secondary, tertiary, or quaternary structures, which results in the related proteins losing their functions or having different activities (8). Therefore, this study aims to elucidate for the first time how high-calorie diets affect the protein secondary structure profile of longissimus dorsi skeletal muscle tissue of inbred C57BL/6J mice using FTIR spectroscopy.

FTIR spectroscopy is a particularly useful method for studying the conformational changes and the secondary structure of proteins (9,10). It is a technique used to analyse the vibrations of functional groups in molecules by exposure to electromagnetic radiation at infrared wavelengths (11). Changes in the vibration of chemical bonds in the infrared region and their absorption properties lead to the formation of spectral peaks. Each of the functional groups has its own frequency of vibration, and each infrared spectrum is specific (12). The many benefits of FTIR include its ability to examine samples at the molecular level without damaging biological systems, straightforward sample preparation procedures, and simultaneously obtain information about all macromolecules in the system (10,13). Since pathological conditions create structural and functional modifications in the biological systems' molecules, these alterations result in changes in vibrational energy levels that can be monitored using FTIR spectroscopy (14). This study will give us an idea of whether high-

calorie diets cause structural changes in protein at the molecular level or not. Hence, it will serve as a guide for researchers conducting current and future human research investigations, as well as contribute to the literature for the diagnosis and treatment of obesity.

MATERIALS AND METHODS

Animals and diets

Four-week-old female inbred C57BL/6J mice were obtained from the Medipol University Mouse House (Istanbul, Turkey) and bred in the facility of Mouse House at Uskudar University for 24 weeks. All animal experiments were confirmed by the Uskudar University Animal Research Local Ethics Committee (approval no. 2017/02). Mice were hosted in groups of five in cages at room temperature (22 ± 2°C) with a 12:12 hour light and dark cycle. During the experiment, the animals had ad libitum access to water and diets. They were divided randomly into three groups, namely the low-fat and low-carbohydrate diet group (control, n = 9), the high-carbohydrate and low-fat diet group (HCD, n = 9), and the high-fat and low-carbohydrate diet group of mice (HFD, n = 5). After weaning at the age of four weeks, all of the mice were given a control diet for a week. After that, high-fat and high-carbohydrate-containing diets were implemented, and this treatment was continued for six months. The control group was fed a low-fat and carbohydrate diet with 25% fat, 20% protein, and 55% carbohydrate (≈ 9% fat by weight) of total calories (S9101-E010, Ssniff Spezialdiäten GmbH, Soest, Germany). The HFD group of mice was given a diet consisting of 45% fat, 20% protein, and 35% carbohydrates (≈ 23% fat by weight) of total calories (S9101-E012, Ssniff Spezialdiäten GmbH, Soest, Germany). Mice in the HCD group were given a diet including 10% of total calories from fat, 20% from protein, and 70% from carbohydrates (≈ 5% fat by weight) (S9101-E014, Ssniff Spezialdiäten GmbH, Soest, Germany). All diets comprise corn starch, maltodextrin, and sucrose as carbohydrates and soybean oil, coconut oil, and tallow as fat. Furthermore, the mineral and vitamin ingredients of the diets were adjusted to meet the requirements of the mice and did not vary between the groups. Animals were weighed every week starting from 4 weeks of age, taking into account their day of birth. All mice were decapitated under anesthesia at the end of 24 weeks, and their skeletal muscle tissues were collected and stored at -80 ^{o}C until further examinations.

Sampling for ATR-FTIR spectroscopy and data analysis

The ATR-FTIR spectra of skeletal muscle tissues were obtained using a Bruker Invenio S ATR-FTIR spectrometer (Germany). The background spectrum was obtained by recording the spectrum of air and automatically subtracted using the Opus 8.5 software program. The muscle samples' spectra were collected at room temperature within the range of 4000-400 cm^{-1} mid-infrared region with 64 scans at 4 cm^{-1} resolution. Under the same circumstances, each tissue was scanned three times from randomly chosen fractions, all of which produced identical spectra. The data and statistical analysis were conducted using the average spectra

of these three replicates. The gathering and manipulation of spectral data were obtained by the same software program (Opus 8.5).

Amide I band was taken into consideration to investigate high-calorie diet-induced alterations in the protein secondary structure of muscle tissues. Firstly, the second derivatives of all spectra were collected using a nine-point Savitzky-Golay smoothing filter to eliminate noise, followed by vector normalization in the 1800-1000 cm^{-1} range. The peak minimums of the second derivative spectra, which are equal to the maximums of the original absorption spectra, were used to calculate peak intensities.

Statical analysis

The results of our study were presented as means ∓SEM. The Kruskal-Wallis one-way ANOVA test was applied using GraphPad Prism 8.02 (GraphPad Software, Inc.) to analyze the experimental data. A p-value less than or equal to 0.05 was considered statistically significant, and *p<0.05, **p<0.01, and ***p<0.001 denoted the level of significance.

RESULTS

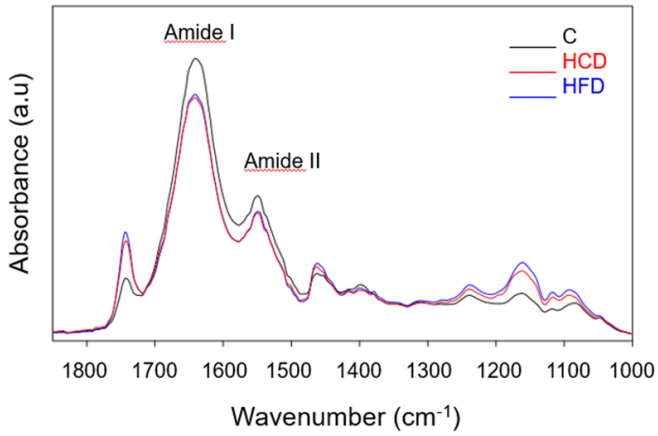

Figure 1. The representative FTIR spectra of skeletal muscles of the HCD, HFD, and control groups in the 1800-1000 cm^{-1} fingerprint region. Baseline offset transforms were performed over the entire spectral range (4000-400 cm^{-1}).

Figure 1 displays the absorbance of skeletal muscle tissues of the control, HCD, and HFD groups in the 1800-1000 cm^{-1} fingerprint region. The bands at 1640 cm^{-1} and 1550 cm^{-1} are assigned to amide I and amide II, which are characteristic vibrations of structural proteins, respectively. The amide I band results from mainly C=O stretching (80%) and C–N stretching vibrations of the protein backbone, and amide II originates from N–H bending (60%) and C–N stretching (40%) vibrations of the proteins, respectively (10). Therefore, the changes in area and/or intensity of these bands refer to changes in the protein structures of samples (12). As seen in Figure 2A, there was a marked decrease in the area ratio of the amide I / amide II bands in the HCD ($p<0.05$)

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and HFD ($p < 0.05$) groups compared to control groups, indicating an alteration in protein structure. In addition, a remarkable decrease in the protein concentration is found only in the HFD ($p<0.05$) groups compared to the control by monitoring the area ratio of the amide I / amide I + amide II bands (Figure 2B).

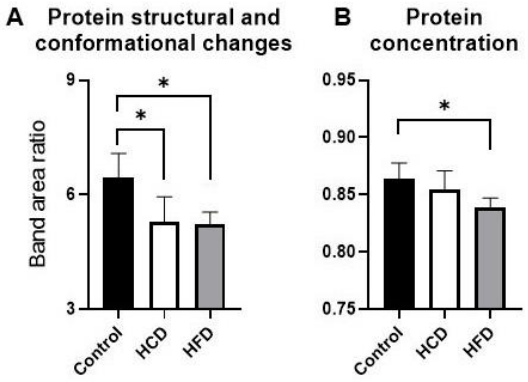

Figure 2. Changes in the band area ratio of (A) protein structural and conformational changes (amide I / amide II), (B) protein content (amide I / amide I + amide II) from the skeletal muscle spectra of the HCD, HFD, and control groups.

Comprehensive information regarding a protein's secondary structure can be determined by analyzing the amide I band (1700-1600 cm^{-1}) using second derivative spectra. Because these spectra reveal sub-bands, which are absent in the original absorbance spectrum (9). Sub-bands in the skeletal muscle tissues' second derivative spectra are observed at 1694, 1680, 1662, 1648, and 1630 cm^{-1}, respectively.

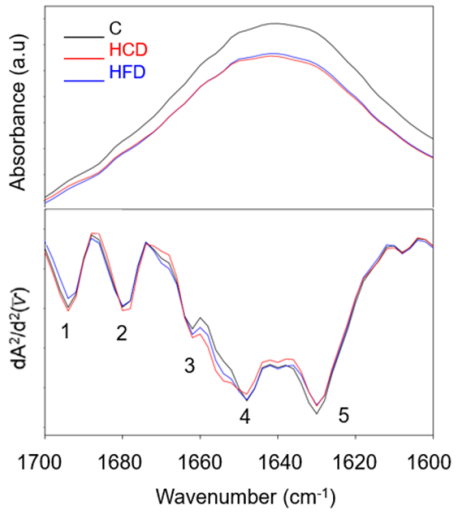

Figure 3. (A) FTIR absorbance spectra and (B) Amide I band second derivative spectra for skeletal muscles of control, HCD, and HFD in the 1700-1600 cm^{-1} region. The second derivative + vector normalization was carried out in the 1700-1600 cm^{-1} range.

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Figure 3 displays the average absorbance spectrum (A) and the vector normalized second derivative spectrum (B) containing the sub-bands of the amide I band. The peak located at 1694 cm^{-1} can be attributed to the presence of an antiparallel β-sheet structure (15,16). The sub-bands at 1680 cm^{-1}, 1662 cm^{-1}, and 1648 cm^{-1} arise from aggregated β-sheet structure (9), β-turn (16,17), and α-helix (16-18). The band at 1630 cm^{-1} often gives information about parallel β-sheet structure (15).

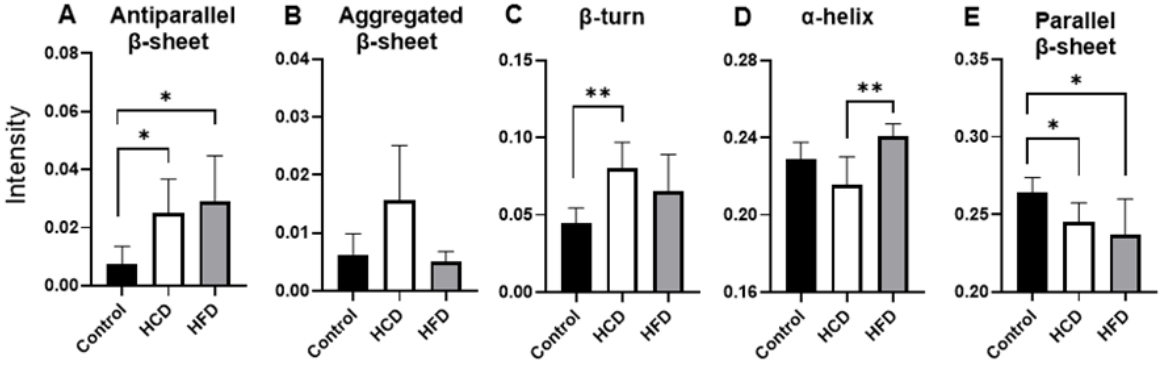

Figure 4. Comparison of the intensities of the main protein secondary structures for (A) antiparallel β-sheet (1694 cm^{-1}), (B) aggregated β-sheet (1680 cm^{-1}), (C) β-turn (1662 cm^{-1}), (D) α-helix (1648 cm^{-1}), and (E) parallel β-sheet (1630 cm^{-1}), for control, HCD, and HFD groups. The level of significance was denoted as *p≤0.05; **p≤0.01.

As depicted in Figure 4, the intensity of the antiparallel β-sheet band (1694 cm^{-1}) increased significantly in the HCD ($p<0.05$) and HFD ($p<0.05$) groups in comparison to the control group. While not statistically significant, the HCD group showed an increased intensity in aggregated β-sheets (1680 cm^{-1}) content. However, in the HFD group, no alteration is found in this content. The intensity of β-turn structure (1662 cm^{-1}) is increased markedly in the HCD group ($p<0.01$), whereas the HFD group demonstrated an insignificant increase in this content compared to the control group. A considerable increase is found in α-helix structure (1648 cm^{-1}) in the HFD group regarding the HCD group. This content insignificantly decreased in the HCD group and slightly increased in the HFD group in contrast with the control group. In addition, β-sheet structure (1632 cm^{-1}) is found to be decreased in both diet groups compared to the control group.

DISCUSSION

The preservation of skeletal muscle mass relies on a fine balance between protein synthesis and breakdown (1) and is affected by diet (19). Therefore, in the current study, we concentrated on the impact of a high-fat and/or high-carbohydrate diet specifically on the protein secondary structure of skeletal muscle tissue. The area ratio of the main protein bands, amide I / amide I + amide II bands, decreased in HFD groups, indicating that there was a decrease in the protein content of the skeletal muscle. A reduction in protein content may indicate a decrease in protein synthesis. This finding is compatible with the results of previous studies in the literature. Deldicque et al. (20) investigated how endoplasmic reticulum stress caused by a high-fat diet

affects the activity of mTORC1 and protein synthesis in muscle cells. They discovered that a high-fat diet causes the protein unfolding response to be triggered in mouse skeletal muscle while also reducing protein synthesis and mTORC1 activity. Anderson et al. (21) used C57BL/6J mice to test the idea that obesity slows down the activation of tissue-specific protein production following food intake. They showed that a high-fat diet did not change the basal rates of protein synthesis in skeletal muscle, but it did slow down protein synthesis activation and change the way tissue-specific protein metabolism works. It has also been reported that the later stage of obesity development in rats fed with a high-fat, high-sucrose diet is characterized by a decrease in protein synthesis rate and an increase in lipid accumulation in glycolytic muscles (22). Although alterations in protein synthesis are crucial to the diagnosis and therapy of disease, there is insufficient knowledge regarding the links between functional qualities such as the structure and dynamics of lipids and proteins in tissues. Variations in gene expression or protein synthesis are not the sole determinants of structural and functional modifications to proteins. Variations in the secondary structures of proteins that occur without an alteration in their expression can also result in protein malfunction (23). Determining the structural changes in proteins and lipids is essential for better understanding the functional anomalies that arise in different disease states and to facilitate the development of new therapeutics (24).

FTIR spectroscopy is one of the most effective experimental methods for determining protein secondary structure. By taking the second derivative of the FTIR spectra, sub-bands of biomolecules can be visualized. The main bands that represent protein structural and conformational changes are amide I and amide II (9). The amide I absorption band, as opposed to the amide II absorption, is more helpful for determining the protein's secondary structure. This is likely because the amide I band effectively arises from just C=O functional groups of the peptide linkages (16). The α-helix and the β-sheet are two folds that are extremely common in protein secondary structures. From the outside, α-helixes and β-sheets look the same, with the exception that carbonyl oxygens are located on one side of a strand while NH groups are located on the other. This gives α-helixes and β-sheets different physical properties. Both mutations in the primary structure of amino acids that comprise a protein and extraordinary circumstances that cause the proteins to denature can disrupt the protein's secondary structure (25). The results of this study discovered that the muscle protein secondary structure profiles of both HCD and HFD groups are impaired. The HCD feeding significantly increased antiparallel β-sheets and β-turn contents and decreased parallel β-sheets content. Moreover, insignificant increases in aggregated β-sheets and a decrease in α-helix content are observed in this group. It is known that the alpha-helix decreases and the beta-sheet increases in the early phases of diseases like diabetes and neurodegenerative diseases (26). We observed similar facts in the HCD group as described above. However, antiparallel β-sheet structure increased markedly, and parallel β-sheets decreased notably, together with insignificantly increased α-helix and β-turn in the HFD group. These modifications may arise from the expression of novel types of proteins with an alpha-helix structure, or they may result from structural reorganizations of pre-existing proteins. Proteins with β-turns are often associated with highly ordered protein

structures, while those with β-sheets and random coils are characteristic of flexible and open structures (27). The increased β-sheet region is linked to an increase in protein-protein interactions among exposed hydrophobic parts, resulting in intermolecular β-sheet structures (28). Therefore, in the HCD group, increased contents of antiparallel β-sheet and aggregated β-sheet may indicate the formation of cross-β-sheet structure. Furthermore, taking into consideration these results together with the decreased α-helix content, it seems that protein aggregation and/or protein-protein intermolecular interaction may be occurring (29). In the HFD group, the structural alteration is less pronounced since the contents of antiparallel β-sheets, β-turn, and α-helix increased and the content of parallel β-sheets decreased. These findings may imply protein rearrangement and/or the formation of intermolecular β-sheet structures. No alteration is observed in aggregated β-sheet content. An increased α-helix and β-turn together revealed a more ordered protein structure in the intact structure of the myofibril. Because the α-helix is a highly prevalent structural motif within the myosin head (>48%) (30), which is responsible for assembling myosin filaments (31). Furthermore, α-helices frequently participate in molecular recognition and protein interactions with other proteins and nucleic acids (32). Thus, stable α-helical structures are important for the maintenance of protein function. Gurbanov et al. (33) also observed an increase in α-helix structures in the brush border membranes of diabetic kidneys. They proposed that this was a result of an increase in the lipid order of the membrane, particularly elevated levels of trans conformers. These trans conformers lead to an expansion in the rigidity and the width of lipid bilayers, consequently amplifying the α-helix transmembrane protein structure. In another study, type I and IV collagens in the bovine Flexor carpi radialis muscle were examined using FT-IR spectroscopy by Petibois et al. (34). They discovered that the relevant quantity of α-helix and triple helix in type I collagen increases while the amount of β-sheets decreases. Most previous studies were generally conducted on high-calorie diet-induced alterations in metabolism and function of muscle tissue (21,35,36). However, there are few studies in the literature examining the secondary structure of skeletal muscle protein using FTIR spectroscopy, but no studies investigating the effects of different diets have been found. Bozkurt et al. (10) revealed that diabetes causes substantial alterations in protein secondary structures in skeletal muscles due to increased aggregated β-sheet structures. Simsek Ozek et al. (37) also reported that low-dose simvastatin causes alterations in the composition, structure, and dynamics of muscle tissue from the extensor digitorum longus of a rat's skeleton. There is a considerable increase in random coil, antiparallel, and aggregated β-sheet structures as well as a considerable decrease in β-sheet structure, which is a sign of protein denaturation. In simvastatin-treated healthy rat liver tissue, Garip et al. (38) reported a significant decrease in α-helix and a rise in random coil, whereas native β-sheet goes down and aggregated β-sheet goes up, indicating simvastatin-induced protein denaturation. Bozkurt et al. (26) examined rat liver proteins' structural changes caused by streptozotocin-induced diabetes and found substantial variations in proteins' secondary structure, including a decrease in the proportion of α-helixes and an increase in the amount of β-sheets, which resulted in protein denaturation and aggregation in the liver of diabetics.

CONCLUSION

As far as we know, this is the first study demonstrating the HCD and HFD effects on the secondary structure of skeletal muscle protein using ATR-FTIR spectroscopy. The findings of the present investigation revealed that protein conformations were affected differently depending on the type of diet. The HCD diet was found to be more detrimental. Elaborated secondary structure analysis of the amide I band revealed the occurrence of intermolecular β-sheet structures and/or protein aggregation in the HCD group, while the HFD group showed structural reorganizations of proteins and/or the formation of intermolecular β-sheet structures. These kinds of structural alterations are critical because any change in protein structure results in alterations in the protein function. In the current study, ATR-FTIR spectroscopy was provided to be a very suitable technique for detecting the secondary structure profile of proteins in muscle tissue.

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Authorship contributions

Concept: A.D, Design: A.D, Data Collection and Processing: N.E.O, Analysis or Interpretation: N.E.O, A.D, Literature Search: N.E.O, A.D, Writing: N.E.O, A.D

Data availibity statement

The datasets utilized and/or examined during the present investigation can be obtained from the corresponding author upon a reasonable request.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Ethics

The experimental protocol of this study was approved by the Uskudar University Animal Research Local Ethics Committee (Approval No: 2017/02).

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