Effect of dihydrotestosterone on gastrointestinal tract of male Alzheimer’s disease transgenic mice

Sritulasi Karri\textsuperscript{a}\textsuperscript{*}, Veronica Acosta-Martinez\textsuperscript{b} & Gopalakrishnan Coimbatore\textsuperscript{c}
\textsuperscript{a}Department of Internal Medicine, Texas Tech University Health Sciences Centre, Lubbock, TX 79430, USA
\textsuperscript{b}USDA-ARS, Cropping Systems Research Laboratory, Lubbock, TX 79415, USA
\textsuperscript{c}The Institute of Environmental and Human Health, Texas Tech University, Lubbock, TX, 79416, USA

Received 18 June 2009; revised 29 January 2010

The cause of Alzheimer’s disease (AD) is still unknown. While research contributions identifying brain as locus of the disease is growing, evidence of severely impaired gastrointestinal (GI) functions with ageing too is accumulating, there is an equal dearth of information on GI tract in AD condition. The aim of this study was to assess the molecular, histological, morphological and microflora alterations of GI tract in male Alzheimer’s transgenic mice. The present study also investigates the effect of dihydrotestosterone (DHT) treatment (1 mg/kg) on AD mice. Histoarchitecture data revealed a significant decrease in the villi number, muscular layer thickness, villi length, width, crypt length, enterocyte length and nuclei length. A shift in colon feces microbial community composition was observed by fatty acid methyl ester analysis. Amyloid precursor protein (APP) expression levels in intestine significantly increased in AD mice revealing its toxicity. DHT treatment attenuated the effect caused by AD on GI morphometrics, APP expression and colon micro flora population. These results for the first time reveal the quantitative and qualitative characteristics of GI tract in male Alzheimer’s disease transgenic mice.

Keywords: Alzheimer’s disease, Amyloid precursor protein, Colon microflora, Dihydrotestosterone, GI tract, Morphometrics

With increasing life expectancy in the world’s population, the physiological changes in the gut and their clinical significance are gaining attention. Gastrointestinal (GI) function is indispensable for maintaining good nutrition. Age-related changes \textit{viz}: malnutrition, impaired gastric motility, bacterial overgrowth, nutrient digestion and absorption, mucosal immune response, and gut neuronal loss are some of the innervations of the GI tract during healthy aging\textsuperscript{1,2}. The pathogenesis of gastrointestinal disorders afflicting the elderly is multifactorial\textsuperscript{2,3} and are different from a younger and healthier population. Many diseases in aging individuals are associated with secondary GI complications and precipitate GI tract disorders \textsuperscript{3,4,9}. The gastrointestinal mucosa forms a barrier against the harsh luminal contents of acid, enzymes, bacteria, and toxins. Disruption of this barrier is the salient feature common to a variety of important gastrointestinal disorders\textsuperscript{7}. The mucosal epithelium of the small intestine rapidly renews and adapts itself after injury or resection\textsuperscript{8}. Crypt cell proliferation leading to intestinal growth and promoting re-establishment of mucosal integrity after injury are essential processes for the differentiation, maintenance, and repair of the intestinal epithelium\textsuperscript{9}.

The normal GI tract microbiota is important for maintenance of host health, providing energy in the form of short-chain fatty acids\textsuperscript{10}, nutrients such as vitamins K and B12\textsuperscript{11}and protection against invading organisms by exerting colonization resistance\textsuperscript{12}. Myriad of microbes colonizing the tract also contribute to and stimulate a variety of host activities besides GI disorders\textsuperscript{13}. The gastro-intestinal tract hosts about 1×10\textsuperscript{12-13} microorganisms, a number close to the number of neurons that comprise the enteric nervous system (ENS). A disturbance in GI-microbial population diversity has been reported as contributing factor to several diseases\textsuperscript{14-16}. Changes in the GI tract, impact of ageing on the intestinal microflora, and prospects for a healthy gut microflora in older people, are well documented\textsuperscript{15}.

Reports suggest that several microbes affect biological responses to steroid hormones and are also capable of synthesizing steroids\textsuperscript{18-20}. Mammalian...
intestinal mucosa reduces testosterone to a variety of 5α-reduced metabolites\textsuperscript{21-26}, including the more potent\textsuperscript{27,28} metabolite of testosterone found in androgen target organs\textsuperscript{29,30}—the dihydrotestosterone. In addition, 5α-reduction of testosterone to dihydrotestosterone may be necessary before testosterone can exert its stimulatory effects on intestinal epithelial cell proliferation\textsuperscript{31,32}. Testosterone or DHT has been shown to be effective in stimulating intestinal contractile and metabolic activities in the epithelium\textsuperscript{33-35}.

Although, Alzheimer’s disease (AD) is one of the leading debilitating diseases in the aged population, either its cause or an effective treatment has not yet been identified. Depletion of steroid hormones associated with normal aging has been identified as a significant risk factor for the development of AD in men\textsuperscript{36,37}. Several age-related changes in the brain pathology of humans have been reported to have an impact on development of gastrointestinal symptoms in the elderly. Significantly, gut has also been considered as the second brain and is known to influence brain directly\textsuperscript{38,39}. Gut amyloidosis has been described\textsuperscript{40-44}. Amyloid precursor protein too was found exclusively in absorptive epithelial cells of the small intestine\textsuperscript{45}.

Alzheimer’s disease is a multisystem disease affecting many systems outside CNS\textsuperscript{46-48}. While investigating the systemic outcome of Alzheimer’s disease condition, Karri et al.\textsuperscript{49} recently identified amyloid protein/gene expression and disrupted alveoli of lungs caused by Alzheimer’s disease. GI tract is a vital organ for extracting nutrients and expelling the waste. It also plays a prominent part of the immune system in addition to keeping microorganisms at bay. To further explore the systemic effects of AD in humans, specifically in the GI tract, the knowledge of changes in GI tract of Alzheimer’s disease animal model is indispensable. In the present study, GI tract health and the effect of DHT treatment, using Alzheimer’s disease male transgenic mice\textsuperscript{50} were investigated. These transgenic mice develop Alzheimer like β-amyloid deposits in the brain when aged. Therefore, this animal model represents an appropriate tool to study the expression pattern of amyloid protein under pathological conditions.

The focus of the present study is to address (a) if AD causes an impact on GI tract, and (b) if DHT treatment attenuates the impact caused by AD on GI tract. It is hypothesized that the changes caused in brain by Alzheimer’s disease have an impact on its first cousin, i.e., the GI tract. To test this hypothesis, in the present study, the relationship between Alzheimer’s disease and GI tract using AD male transgenic mice has been established. GI tract morphology, histopathology, immunoblotting and fatty acid methyl esters (FAME) analysis techniques were performed to identify Alzheimer’s related changes. Further, the effect of DHT treatment, on the GI tract histoarchitecture, morphometrics, amyloid β precursor protein expression, and colon microbial community composition were analyzed. These data represent the first experimental evidence directly linking the androgen effect on the GI tract in AD animal model.

Materials and Methods

Transgenic mice—Male wild type (WT) and Alzheimer’s disease (AD) hemizygous B6;SJL-Tg(APP\textsubscript{SWE}) 2576Kha transgenic mice\textsuperscript{50}; (12-15 weeks old) were purchased from Taconic Farms, New York. These carry a transgene coding for the 695-aminoacid isofrom of human Alzheimer amyloid precursor protein (APP) derived from a Swedish family with early-onset Alzheimer’s disease. This model has been shown to be useful for APP expression and study of drugs for treatment or prevention of AD. Hence, this model was chosen for the present studies. Upon arrival mice were housed individually with controlled temperature (25°C) and 12 h alternate light-dark cycle. Food and water were provided \textit{ad libitum}. Both WT and AD mice were randomly divided into following 4 groups of 5-8 animals each (Gr.1: WT; Gr.2: WT+DHT; Gr.3: AD; Gr.4: AD+DHT). They were treated subcutaneously (sc) with vehicle or DHT (Sigma; 1mg/kg body weight in 1% ethanol) for 21 days. DHT is regarded as a more potent androgen than testosterone because it has a higher affinity for androgen receptor (AR) compared with testosterone\textsuperscript{51} and DHT-activated AR has a relatively long half-life\textsuperscript{52,53}. In addition, DHT, unlike testosterone, is not aromatized to estrogen\textsuperscript{54} and has been shown to inhibit aromatase activity \textit{in vitro}\textsuperscript{55}. Therefore, DHT was used over testosterone in the present study. The dose of DHT was selected on the basis of previously\textsuperscript{56,57} published and our initial studies. This dose and duration stimulate the weights of classical androgen-sensitive tissues\textsuperscript{49,58,59}. Studies also indicate that the gut is responsible for the failure of oral androgens to provide effective
androgen replacement therapy\textsuperscript{26,60}. Skin is composed of a series of androgen-sensitive structures, steroidogenic enzymes thus providing all the enzymatic machinery necessary for the local synthesis of sex steroids\textsuperscript{61,62}. Hence, subcutaneous injection route was chosen on the basis of above mentioned reports and our initial results. Mice were 23-27 weeks old when they were sacrificed on day 22 of the study. Fecal samples were collected and frozen in -80°C until further analysis. Intestine tissue was either snap frozen in liquid nitrogen for immunoblot or fixed in 4% paraformaldehyde for histopathological analysis. Animal care and experiments were approved and conducted according to the Texas Tech University Health Sciences Centre Institutional Animal Care and Use Committee guidelines.

Body and organ weights—Control and experimental groups of wild type (WT, WT+DHT) and AD (AD, AD+DHT) mice were sacrificed by CO\textsubscript{2} asphyxiation on day 22 post DHT treatment. The body weights were recorded everyday prior to DHT treatment as reported earlier\textsuperscript{49}.

**GI tract morphological changes**—GI tracts from stomach to the distal end were dissected out, cleaned, measured and photographed using a digital camera (Canon Model: Rebel XT).

Histopathology—Tissues were fixed in 4% paraformaldehyde. Paraffin sections (5\,µm thick) were cut and stained with hematoxylin and eosin. Microphotographs were taken using an Olympus microscope BX50 with Nikon digital camera DXm1200. Pictures were captured using ACT-1 Nikon version 2.2 software. Analysis was performed using Metamorph 7.5.4.0 software from Molecular Devices, Downing town, Philadelphia, Pennsylvania. Photo editing software Adobe Photoshop CS2 was used for final processing.

Semi quantitative analysis of morphometrics—Hematoxylin and eosin stained paraffin sections were used for morphometrics analysis. The following parameters were analyzed in all the four experimental groups: villi number, muscular layer thickness, crypt length/depth, villi length, villi width, enterocyte length and enterocyte nucleus length. Image analysis was carried out using software provided by Metamorph 7.5.4.0 2008 from Molecular Devices, Downing town, Philadelphia, Pennsylvania.

Immunoblotting—Intestine was homogenized using microprobe, polytron setting of “7”, 30 sec (10 sec each time) on ice in lysis buffer (10 mM Tris, 250 mM sucrose; 0.779 mM EDTA, pH 7.4) containing 10 mg/mL aprotinin and 1 mM phenylmethylsulfonyl fluoride (PMSF) (Sigma). Equal amounts of protein (50 µg), as determined by the BCA method\textsuperscript{63} was loaded onto one-dimensional electrophoresis on 12% polyacrylamide gels containing 0.15 M sodium dodecyl sulphate (SDS-PAGE), electro transferred to polyvinylidenedifluoride (PVDF) membrane (Bio-Rad) in transfer buffer and nonspecific binding was blocked with 4% nonfat milk for 3 h at 4°C in phosphate buffered saline containing 0.05% Tween 20 (Sigma). Membranes were then incubated overnight at 4°C with APP (1:10,000) or \(\beta\)-actin (1:7500) primary antibodies. After washing the membranes and incubating at 4°C with anti-rabbit (1:20,000) or anti-mouse (1:2500) antibodies respectively, bands were visualized using chemiluminescence (Pierce) and exposure to X-ray film (Kodak). Band densities on X-ray films were quantified using the Alpha imager 2000 (Alpha Innotech Corp., San Leandro, CA, USA). The bands of interest were designated manually and the software then calculated the integrated density value \([\text{IDV} = \Sigma (\text{each pixel value – background})]\) within the designated area.

Fatty acid methyl ester (FAME) analysis—The mice stool samples (0.25 g) were mixed in a vortex for 5-10 sec in a 15 ml glass tube containing 1.0 ml methanolic base (13% NaOH, 43% methanol). The samples were placed in a water bath at 100°C for 5 min and again for 25 min to release the fatty acids from the microbial phospholipids. After cooling, the samples were incubated in a water bath at 80°C for 10 min with 2 ml methylation reagent (54% 6N HCl, 46% methanol) to convert the fatty acids to fatty acids methyl esters (FAMEs). Then 1.25 ml of extraction reagent (50% hexane, 50% methyl-tert-butyl ether) was added to remove the fatty acid methyl esters from the aqueous phase. Following this, the upper solvent phase with fatty acid esters was transferred to a new preparation glass tube. Remaining fatty acids in the aqueous phase were removed with 3 ml mild base solution (10.8% NaOH) and centrifuged at 2000 rpm. The upper organic solvent phase was transferred to gas chromatography (GC) vials and the samples were analyzed in a 6890 GC Series II (Hewlett Packard, Wilmington, DE) equipped with a flame ionization detector and 25m \(\times\) 0.2mm fused silica capillary column using ultra high purity hydrogen as the carrier gas. The temperature program was ramped from
170°–250°C at 5°C min⁻¹.

Fatty acids were identified and their relative peak areas (percent) were determined with respect to the other fatty acids in a sample using the MIS Aerobe method of the MIDI system (Microbial ID, Inc., Newark, DE). All peaks in a sample were compared to standard fatty acids provided by MIDI (Microbial ID, Newark, DE). The FAME designations are described by the number of carbons (C), followed by a colon, the number of double bonds and then by the position of the first double bond from the methyl (ω) end of molecules, and cis and trans isomers are indicated by ‘c’ or ‘t’, respectively. Branched fatty acids are indicated by the prefixes ‘i’ and ‘a’ for iso and anteiso, respectively.

Statistical analysis—Prism software (version 4.02, Graph Pad Inc., San Diego, CA, USA) was used for graphical presentation and statistical analysis. Data are presented as standard error mean (SEM) values of ‘n’ independent experiments with variability given as mean±SE. Analyses of variance (ANOVA) performed included one-way ANOVA for matched samples followed by Newman-Keuls post-hoc test of differences between all group means. P<0.05 was considered statistically significant.

Results
Gross morphology of gastrointestinal (GI) tract—The morphological appearance of total GI tract length of mice in the four groups, wild type (WT), Alzheimer’s disease (AD), wild type treated with dihydrotestosterone (WT+DHT) and Alzheimer’s disease treated with DHT (AD+DHT) is shown in Fig. 1. WT, AD and WT+DHT GI tracts were photographed by folding the GI tract after measuring, while the AD+DHT GI tract is stretched to show an

Fig. 1—Photographs showing morphology of gastrointestinal tract (GIT). WT= wild type, AD= Alzheimer’s disease, DHT= dihydrotestosterone. Note the change in the GIT length in AD and AD+DHT groups compared to WT and WT+DHT groups (A). (B) length of GIT in (inches) and (C) weights of GIT/100g body weight. Note a non-significant increase of GIT weight in AD group. Data are mean±SE from 5-8animals in each group. P values: <0.001: *vs WT, †vsAD; ‡vs WT+DHT.
increase in length compared to AD group (Fig.1A). A significant ($P<0.001$) decrease in the total length of the GI tract in AD and AD+DHT groups, when compared to both WT and WT+DHT was observed. There was a significant ($P<0.001$) increase in GI tract length caused by DHT treatment to AD group compared to untreated AD group (Fig.1B). A non-significant increase of GI tract weight in AD group was observed (Fig.1C). Further, we also observed rubbery appearance of GI tracts in both AD and AD+DHT groups.

Impaired changes in small intestine light microscopic structure—Image analysis of hematoxylin and eosin stained WT intestine sections revealed well-developed muscular layer, crypt layer and villi (Fig. 2, A1, B1). AD mice intestine revealed reduced muscular layer, crypts, and villi infiltrated with lymphocytes, plasma cells, eosinophils, diffused, blunt ended, flattened and disrupted (Fig. 2, A2, B2). Exogenous DHT treatment to WT mice showed normal intestinal architecture as observed in untreated WT (Fig. 2, A3, B3). On the other hand, histological evaluation of AD intestine treated with DHT showed a few well-developed villi compared to untreated AD group. Overall, the histoarchitecture after DHT treatment of AD mice was not restored to normalcy (Fig. 2, A4, B4). Bar graph illustrates villi number fold change after DHT treatment in WT and AD mice. A significant ($P<0.01$) decrease in villi number was observed in AD group compared to WT and WT+DHT. DHT treatment increased villi number significantly ($P<0.01$) in WT+DHT compared to WT. Also, a significant ($P<0.001$) increase in villi number was observed after DHT treatment in AD mice compared to untreated AD group. However, villi number remained significantly low in AD+DHT group, when compared to both WT ($P<0.01$) and WT+DHT ($P<0.001$) groups.

Differential changes in morphometrics of AD mice GI tract — In WT and WT+DHT groups, villi show
normal, well-developed structure from the muscular layer upwards to the peak of the villi (Fig. 3). Crypt layers are well arranged at the base of the villi. Healthy epithelial cells are aligned along the walls of the villi. Stroma looks healthy with lymphatic capillaries. Brush border smoothly lines the epithelial cells along the sides of the villi to form a well-defined peak. In AD and AD+DHT groups, abnormalities are observed from the muscular layer to the villi peak viz; thinning of muscular layer, disruption of crypts, microscopic hemorrhage, capillary congestion, lymphatic dilation and inflammatory cells, lymphocytes, plasma cells, eosinophils and also infiltration in the stroma was observed. Villi appeared short and fused and lost their normal arrangement. Epithelial compression and villi destruction is observed in both groups.

Muscular layer thickness: A significant ($P<0.001$) decrease in muscle layer thickness of AD group compared to WT and WT+DHT group was observed (Fig. 3B). DHT treatment caused a significant ($P<0.001$) increase in muscle layers of the WT+DHT and AD+DHT groups when compared to WT and AD untreated groups respectively. However, in AD+DHT group the muscular layer thickness was significantly ($P<0.001$) thinner when compared to WT and WT+DHT groups.

Crypt length/depth: A significant ($P<0.001$) decrease in crypt length was observed in AD group compared to WT and WT+DHT groups (Fig. 3C). While a significant ($P<0.001$) increase was observed in AD+DHT compared to AD group. However, the

![Figure 3](image-url)
increase did not bring the crypt length to normalcy. Crypt length remained significantly ($P<0.001$) small in AD+DHT compared to WT and WT+DHT groups.

Villi length: Compared to WT and WT+DHT groups, villi length was significantly ($P<0.001$) reduced in AD and AD+DHT groups (Fig. 3D). On the other hand, a significant ($P<0.001$) increase in villi length was observed in AD+DHT group compared to AD group.

Villi width: Significant ($P<0.001$) villi width reduction was observed in AD and AD+DHT groups compared to WT and WT+DHT groups (Fig. 3E). However, DHT treatment to AD group had no effect on villi width.

Higher magnification of villi showing enterocytes and nuclei: In WT and WT+DHT groups, enterocytes and nuclei were arranged in a healthy fashion and enterocytes lead to a fine brush border giving shape to the villi (Fig. 4A). In AD and AD+DHT groups, brush border was not smoothly arranged; infiltration of cells in enterocyte region, diffusion of enterocytes and nuclei and distortion of enterocytes and nuclei morphology were observed.

Enterocyte length: A decrease in enterocyte length was observed in AD and AD+DHT groups compared to WT ($P<0.01$) and WT+DHT ($P<0.001$) groups. (Fig. 4B). DHT treatment failed to bring back the enterocyte length to normalcy in AD group.

Enterocyte nucleus length: A reduction in the enterocyte nucleus length in AD and AD+DHT groups compared to WT ($P<0.01$) and WT+DHT ($P<0.001$) groups was observed (Fig. 4C). DHT treatment to AD failed to bring back the nuclei length.

Fig. 4—Photomicrographs of villi showing changes in enterocyte and nucleus after DHT treatment. Bar=25µm [H&E]. WT= wild type, AD=Alzheimer’s disease, DHT= dihydrotestosterone. Note the distorted enterocyte and nucleus in AD and AD+DHT villi groups compared to untreated WT & AD (A). Bar graphs showing changes in enterocyte (B) and nucleus (C) length after DHT treatment in WT & AD mice. Data are mean±SE from 5-8 animals in each group. $^aP<0.01$ vs WT, $^cP<0.001$ vs WT+DHT.
Evidence of amyloid precursor protein (APP) expression by immunoblotting—To examine whether Alzheimer’s disease marker amyloid precursor protein is expressed in AD intestine, immunoblot analysis was performed. A significant (\(P<0.001\)) increase in APP expression in AD group compared to WT was observed (Fig. 5). DHT treatment to AD group significantly (\(P<0.001\)) reduced APP expression compared to untreated AD. However, AD+DHT group APP levels remained significantly (\(P<0.001\)) higher when compared to WT and WT+DHT groups. The bar graph depicts the mean of % integrated density value (IDV) normalized to \(\beta\) actin a housekeeping gene.

Effect of Alzheimer’s disease on colon microbial community composition—FAME analysis revealed 5-34 fatty acids extracted from the colonic stool samples depending on the experimental group (unpublished data). The major differences in FAME peaks in the four groups are presented in Table 1. The data revealed FAMEs within saturated, unsaturated, branched, methylated, hydroxy, cyclo, alcohol, and unknown groups. Indicators were recognized as general microbes, gram-negative bacteria (G-), fungi, protozoa, gram-positive bacteria (G+) and actinomycetes. An experimental group where FAME peak was not detected (ND) and where just one sample (1) was detected in a group is represented in parenthesis. Only FAMEs showing significant differences between groups are presented (in bold number) in the table. General microbes represented by indicator 16:0 decreased significantly (\(P<0.05\)) in WT+DHT compared to WT group. The FAME 18:0 significantly (\(P<0.05\)) increased in WT+DHT and AD+DHT groups compared to WT and AD groups. Gram-negative bacteria represented by FAMEs 18:1\(\omega6\)c and 18:1\(\omega7\)c increased significantly (\(P<0.05\)) in WT+DHT compared to AD. Protozoa represented by indicator 20:4\(\omega6\) revealed a significant (\(P<0.05\)) increase in WT+DHT and AD+DHT compared to WT & AD groups. Gram+positive bacteria represented by \(i14:0\) revealed a significant decrease (\(P<0.05\)) in WT+DHT compared to WT and AD groups. Indicator 18:03OH revealed a significant (\(P<0.05\)) increase in WT+DHT.
KARRI et al. : DIHYDROTESTOSTERONE EFFECT ON ALZHEIMER’S GI TRACT

Discussion
Evidence is accumulating that AD is a multisystem disease affecting many systems outside the CNS. In this communication a relationship between Alzheimer’s disease and GI tract using AD male transgenic mice as model is reported. The results explicitly show considerable alterations in GI tract morphology, morphometrics, amyloid protein expression and shift in colon fecal microflora, in Alzheimer’s disease condition. Further, upon dihydrotestosterone treatment for 3 weeks, these alteration -caused by AD were partially restored to normalcy. Taken together, the present results establish a

Table 1—Comparison of fatty acid methyl esters (FAMEs) of microflora in colon fecal samples after DHT administration in WT and AD mice
[Values are mean±SE from 5-6 animals in each group]

<table>
<thead>
<tr>
<th>FAME Organism</th>
<th>Sum of diversity index Gr. 1 (WT)</th>
<th>Gr. 2 (AD)</th>
<th>Gr. 3 (WT+DHT)</th>
<th>Gr. 4 (AD+DHT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:0</td>
<td>General microbes</td>
<td>0.25±0.15</td>
<td>0.24±0.17</td>
<td>0.27±0.02</td>
</tr>
<tr>
<td>14:0</td>
<td>General microbes</td>
<td>2.0 ± 0.16</td>
<td>1.7 ± 0.15</td>
<td>1.2 ± 0.16</td>
</tr>
<tr>
<td>15:0</td>
<td>General microbes</td>
<td>1.27 ± 0.24</td>
<td>0.95 ± 0.08</td>
<td>0.58 ± 0.09</td>
</tr>
<tr>
<td>16:0</td>
<td>General microbes</td>
<td>24.08 ± 0.63</td>
<td>22.44 ± 0.89</td>
<td>a 19.45 ± 0.87</td>
</tr>
<tr>
<td>17:0</td>
<td>General microbes</td>
<td>1.05 ± 0.17</td>
<td>0.74 ± 0.11</td>
<td>0.84 ± 0.07</td>
</tr>
<tr>
<td>18:0</td>
<td>General microbes</td>
<td>7.17 ± 0.24</td>
<td>6.93 ± 0.36</td>
<td>ab 19.10 ±2.78</td>
</tr>
<tr>
<td>20:0</td>
<td>General microbes</td>
<td>0.68 ± 0.03</td>
<td>0.51 ± 0.11</td>
<td>ND</td>
</tr>
<tr>
<td>Unsaturated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:1(12-13) Gram – bacteria</td>
<td>0.120 (1)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>15:1 ω 6c Gram – bacteria</td>
<td>0.56 ± 0.13</td>
<td>0.38 ± 0.059</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>17:1 ω 8c Gram – bacteria</td>
<td>0.65 ± 0.21</td>
<td>0.51 ± 0.25</td>
<td>0.30 ± 0.05</td>
<td>ND</td>
</tr>
<tr>
<td>18:1 ω 6c Gram – bacteria</td>
<td>3.29 ± 0.68</td>
<td>2.26 ± 0.28</td>
<td>b 5.35 ± 1.08</td>
<td>15.19 (1)</td>
</tr>
<tr>
<td>18:1 ω 7c Gram – bacteria</td>
<td>2.306 ± 0.08</td>
<td>1.915 ± 0.21</td>
<td>b 3.080 ± 0.31</td>
<td>2.481 ± 0.10</td>
</tr>
<tr>
<td>18:1 ω 9c Gram – bacteria</td>
<td>20.28 ± 1.41</td>
<td>21.67 ± 0.80</td>
<td>14.60 ± 1.7</td>
<td>14.61 ± 3.01</td>
</tr>
<tr>
<td>18:3 ω 6c Fungi</td>
<td>0.48 ± 0.11</td>
<td>0.50 ± 0.16</td>
<td>0.77 ± 0.29</td>
<td>0.43 ± 3.0</td>
</tr>
<tr>
<td>19:1 Gram – bacteria</td>
<td>0.56 ± 0.09</td>
<td>0.55 ± 0.06</td>
<td>ND</td>
<td>1.66(1)</td>
</tr>
<tr>
<td>20:1 ω 9c Gram – bacteria</td>
<td>0.56 ± 0.09</td>
<td>0.55 ± 0.06</td>
<td>ND</td>
<td>1.66(1)</td>
</tr>
<tr>
<td>20:2 ω 6,9c Gram – bacteria</td>
<td>0.56 ± 0.09</td>
<td>0.55 ± 0.06</td>
<td>ND</td>
<td>1.66(1)</td>
</tr>
<tr>
<td>20:4ω6 Gram – bacteria</td>
<td>0.29 ± 0.06</td>
<td>0.34 ± 0.02</td>
<td>ab 3.53 ±0.18</td>
<td>a,b4.82 ±1.3</td>
</tr>
<tr>
<td>Branched</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i4:0</td>
<td>Gram + bacteria</td>
<td>0.22 ± 0.013</td>
<td>0.25±0.0</td>
<td>ab 0.14 ±0.01</td>
</tr>
<tr>
<td>i5:0</td>
<td>Gram + bacteria</td>
<td>0.80 ± 0.14</td>
<td>0.77±0.23</td>
<td>0.56 ± 0.08</td>
</tr>
<tr>
<td>a1:5ω6c Gram + bacteria</td>
<td>1.10 ± 0.23</td>
<td>1.24±0.32</td>
<td>1.23 ± 0.21</td>
<td>1.11(1)</td>
</tr>
<tr>
<td>i6:0</td>
<td>Gram + bacteria</td>
<td>0.15 ± 0.01</td>
<td>ND</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>i7:0</td>
<td>Gram + bacteria</td>
<td>0.32 ± 0.05</td>
<td>0.42±0.13</td>
<td>ND</td>
</tr>
<tr>
<td>a1:7ω6c Gram + bacteria</td>
<td>0.39 ± 0.00</td>
<td>0.41(1)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>i9:0</td>
<td>Gram + bacteria</td>
<td>ND</td>
<td>ND</td>
<td>0.43 ± 0.057</td>
</tr>
<tr>
<td>i2:0</td>
<td>Gram + bacteria</td>
<td>ND</td>
<td>1.107 (1)</td>
<td>ND</td>
</tr>
<tr>
<td>methylated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10Me17:0 Actinomycetes</td>
<td>ND</td>
<td>ND</td>
<td>0.319 (1)</td>
<td>ND</td>
</tr>
<tr>
<td>Hydroxy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0 2OH</td>
<td>ND</td>
<td>0.22 (1)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>16:0 2OH</td>
<td>ND</td>
<td>0.22 (1)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>16:0 3OH</td>
<td>ND</td>
<td>0.22 (1)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>i17:0 3OH</td>
<td>1.61 ± 0.08</td>
<td>ND</td>
<td>0.22 (1)</td>
<td></td>
</tr>
<tr>
<td>18:0 2OH</td>
<td>ND</td>
<td>0.22 (1)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>18:0 3OH</td>
<td>0.26 ±0.01</td>
<td>0.32 ± 0.0 (1)</td>
<td>a1.77 ± 0.36</td>
<td>1.39 ± 0.43</td>
</tr>
<tr>
<td>Cyc Cyclo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:0 CYCLO Gram - bacteria</td>
<td>ND</td>
<td>ND</td>
<td>0.402 (1)</td>
<td>ND</td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:0 N alcohol</td>
<td>ND</td>
<td>ND</td>
<td>0.908 (1)</td>
<td>0.97(1)</td>
</tr>
<tr>
<td>unknown14_959</td>
<td>0.29 ± 0.04</td>
<td>ND</td>
<td>0.28 ± 0.09</td>
<td>ND</td>
</tr>
</tbody>
</table>

WT= wild type, AD=Alzheimer’s disease, DHT= dihydrotestosterone. ND= Not detected. Parentheses (1) represent one sample identified in that group, which is not considered for statistical analysis. P<0.05; a vs WT, b<0.05 vs AD.

compared to WT group.
relation between Alzheimer’s disease, GI tract and androgen treatment.

The external appearance of the GI tract was studied to determine the AD caused changes in morphology and the effect of DHT treatment. The most obvious morphological alteration was shorter length of the AD GI tract compared to WT, which was accompanied by a decreased luminal diameter of the GI tract in AD mice (unpublished data). The decreased diameter could be accounted for by a reduction in the muscular layer of AD mice as observed in the histological data. The decreased length could be the result of an enhanced state of tonic contraction of the intestine. Although, statistically not significant, increase in the mass of GI tract could be triggered in some way in response to the impaired GI function in AD mice. Another possibility is that some of the changes are due to the development of the disease itself. The size of GI tract changes with energetic, functional demands and condition of the disease in individuals. 

The mechanisms affecting intestinal length are unknown; however, it can be hypothesized that increased small intestinal length in DHT treated AD group is a compensatory response to the decreased absorptive capacity associated with decreased surface area (decreased villi length) and/or to direct competition with the microbiota for dietary nutrients.

Morphometric analysis is a quantitative assessment, which is especially important in the diagnosis of different diseases and pathological conditions. GI tract morphometrics in AD exhibited interesting findings such as: decrease in the mean crypt, enterocyte, nuclei and villi number and length. Villous atrophy and crypt hyperplasia have been used to discriminate between various types of diseases. Villi atrophy in the present study also suggests a decrease in absorption capacity. In addition, morphometrics have been used to evaluate the condition of the intestinal mucosa after treatment, the effectiveness of treatment with diseases, and the influence of environmental factors on mucosal morphology. Hormones are known to play important roles in regulating gastrointestinal secretion, and whole animal physiology. In the present study, an increase in muscular layer thickness by DHT treatment in AD shows that the intestinal smooth muscle differentially responds to physiologically relevant concentrations of androgens. Also, increase in villi and crypt number, enterocyte and villi length, after DHT treatment in the present study corresponds with the testosterone caused increase in the cell growth of the intestine.

In the present study APP levels were significantly higher in AD intestine compared to WT, suggesting that the disease is the cause of such an increased expression. The present study also reveals that DHT treatment in AD down regulated APP expression. However, such levels were not brought back to normalcy. This could also be the reason for persistence of disrupted histopathology and morphometrics in DHT treated AD mice as a result of amyloid toxicity. Recently, substantial amyloid precursor protein was found exclusively in absorptive epithelial cells of the small intestine and was elucidated through histopathological studies. Biochemical and immunoblot blot analysis of intestinal amyloid deposits was also revealed in hemodialysis patients. With an increase in systemic amyloid deposition and visceral organ involvement in amyloidosis, further studies are required to elucidate the amyloid mechanism in Alzheimer’s gut.

Changes in the GI tract, as well as modification of diet and host immune system, inevitably affect the colonic microbiota, thereby changing the bacterial population diversity. The normal intestinal microbiota is important for maintenance of host health, providing energy in the form of short-chain fatty acids and protection against invading organisms by exerting colonization resistance. Researchers have identified specific FAME indicators for different types of bacteria (G+, G- and actinomycetes), fungi and protozoa groups with studies using pure cultures and environmental samples. However, there is paucity of data related to changes in microbial community composition that take place with onset of Alzheimer’s disease. The FAME analysis data of AD and DHT treated group show that there are also changes in the fecal colon microflora. Generally, G+ populations were not affected by AD, but there was a significant shift towards G-population in the microflora of the AD group feces. Overall FAME indicators show a significant increase of general microbial populations including G-bacteria and protozoa with DHT treatment. Microbes are known to affect biological responses to steroid hormones and are also capable of synthesizing steroids. In the present study, shortening of the epithelial layer and villi disruption, as revealed by intestinal pathology, and disruption of
the mucosal barrier, could be additional factors contributing to the changes in the microbial community composition shift as a result of direct competition for dietary nutrients. The gastrointestinal mucosa forms a barrier against the harsh luminal contents of acid, enzymes, bacteria, and toxins. Disruption of this barrier is a salient feature of a variety of common and important gastrointestinal disorders. Thus, DHT treatment partially improved the histoarchitecture of the intestine in AD group by reducing the APP expression and also keeps check on the intestinal microflora. This observation concurs with previous reports.

In summary, to the best of our knowledge, this is the first report of the changes in the GI tract of male Alzheimer’s disease transgenic mice and its response to DHT treatment. Changes caused by AD in GI tract morphology, histoarchitecture, morphometrics, amyloid precursor protein levels and colon microbial community composition were observed. Thus, DHT treatment partially improved the histoarchitecture of the intestine in AD group by reducing the APP expression and also keeps check on the intestinal microflora. This observation concurs with previous reports.

Acknowledgement

Funding support provided by the South Plains Foundation, Lubbock, TX, USA to SK is acknowledged.

References

12. van der Waaij D, Berghuis-de Vries J M & Lekkerkerk L-v, Colonization resistance of the digestive tract in conventional and antibiotic-treated mice, J Hyg (Lond), 69 (1971) 405.


56 Labrie F, Luu-The V, Martel C, Chermomoretz A, Calvo E, Morissette J & Labrie C, Dehydroepiandrosterone (dhea) is an anabolic steroid like dihydrotestosterone (dht), the most potent natural androgen, and tetrahydrogestrinone (thg), J Steroid Biochem Mol Biol, 100 (2006) 52.


