Possible Association of Altered Serum Lipids in Obese Adult Females with Adenoviral Infection

Agnieszka Jaworowska and Grzegorz Bazylak*

Department of Pharmaco-Bromatology & Molecular Nutrition, Faculty of Pharmacy, Collegium Medicum, Nicolaus Copernicus University, Jagiellonska 13, PL-85-067 Bydgoszcz, Poland

Abstract: The aim of this pilot study was to investigate the possible role of adenovirus infection in etiology of obesity. Antibodies (IgG, IgA, IgM) to adenovirus were determined in a total of 71 Caucasian, apparently healthy, middle aged (mean 45.9 ± 13.6 years), living in urban area, cohort of women - including overweight/obese (OW/OB, n = 50) and normal weight (NW, n = 21) group - by using serotype non-specific enzyme linked immunosorbent assay (ELISA). Triglycerides (TG), total- (TChol) and HDL-cholesterol (HDLChol) concentrations in collected serum samples were performed by standard enzymatic methods. The level of LDL cholesterol (LDLChol) was calculated using the Friedewald formula. Anthropometric measurements were taken and body mass index (BMI), percentage of fat mass (%FM) and waist-to-hip ratio (WHR) were calculated. There were no associations ($p > 0.05$) observed between IgG^+ or IgA^+ seropositivity for adenovirus and measured obesity indicators as BMI, %FM, WHR in studied female groups. However, in the group of OW/OB subjects with combined seropositivity (IgG^+/IgA^+) - both for IgG and IgA antibodies to adenovirus - which suggest chronic adenoviral infection the significantly ($p < 0.05$) lower serum TG, TChol and LDLChol concentrations have been observed in comparison with the seronegative (IgG^-/IgA^-) females from the OW/OB and NW group of females. Findings of our pilot study suggest that infection of some adenoviruses may predispose to altered serum lipids in some adult OW/OB females. Further studies should be made to critically evaluate this association and its possible consequences to currently accepted protocols for obesity prevention and treatment. Especially, in this group of OW/OB females, the involvement of measurements of the serotype-specific neutralizing antibodies to adenovirus or isolation/identification of specific adenovirus like particles in serum and adipose tissue using convective interaction media (CIM) monolithic column technology should be advisable.

Keywords: Infectoobesity, lipoproteins, cholesterol, adenovirus, immune disfunction.

1. INTRODUCTION

Adenoviruses were first isolated in 1953 year by Rowe *et al.* [1]. They are medium-sized, non-enveloped, double-stranded DNA viruses that commonly cause respiratory, ocular, gastrointestinal and other diseases in humans [2,3]. To date, 55 different serotypes of adenoviruses that infect human beings have been identified. The various serotypes are grouped into the six species from A to G on the basis of their haemagglutination properties, oncogenic potential in rodents, the percent homology and guanine-cytosine ratios in their DNAs [2,4,5]. Adenoviruses are transmitted primarily by direct contact and by the fecal-oral route. Fomites may be an important mode of spread, because adenoviruses are stable in the environment [2,6]. Natural adenoviruses infections have a worldwide distribution and are common and frequent among all age groups, with a higher frequency reported in children below the age of five years [2, 7]. About 80% of humans are pre-exposed to these viruses and are reported to have high titers of adenovirus neutralizing antibodies [8,9]. Most of these infections are asymptomatic, and symptomatic infections are mild and self-limiting in healthy immunocompetent adults [10]. However, adenoviruses can cause severe diseases in an immunocompromised patients including hepatitis, encephalitis, nephritis, meningoencephalitis and pneumonia [3,11]. Adenovirus are endemic in pediatric populations, but epidemics may occur in populations crowded together, for example acute respiratory disease in military groups, pharyngoconjunctival fever in swimming pools, and epidemic keratoconjunctivitis in medical facilities [2,6,12,13].

It has also been observed that adenovirus promotes adiposity in animals and is casually associated with development of human obesity [14-20]. In humans, the presence of antibodies to human adenovirus Ad-36 in serum is associated with increased body mass index and paradoxical reduction in serum total cholesterol (TChol) and triglyceride (TG) concentrations [17,18]. The prevalence of overweight and obesity (OW/OB) has dramatically increased worldwide, and according to WHO there is a global epidemic of obesity [21,22]. Obesity is a chronic disease with multiple etiology including also such non-dietary factors as a viral and bacterial infection [17,18,20,23-25]. Recently, the evidences on the adenovirus infection related obesity where confirmed in adult patients from USA [26-28], Italy [28], Korea [29] and in pediatric subjects from Korea [30,31], but not fully validated association was observed in studies on Belgium and Dutch obese persons [32,33]. Thus,

*Address correspondence to this author at the Department of Pharmaco-Bromatology & Molecular Nutrition, Faculty of Pharmacy, Collegium Medicum, Nicolaus Copernicus University, Jagiellonska 13, PL-85-067, Bydgoszcz, Poland; Tel: +4852 585 3915; Fax: +4852 585 3817;
E-mail: gbazylak@cm.umk.pl

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the aim of our pilot study was to investigate the possible role of human adenovirus infection in etiology of obesity in the sample cohort of adult female subjects recruited from typical middle-sized urban area in Poland.

2. MATERIALS AND METHODS

The study population comprised a 71 Caucasian, apparently healthy, adult women who were enrolled from Bydgoszcz (Poland) by advertisement in local media between March 2006 and May 2007. None of these subjects: 1) indicate abnormalities in the physical examination, 2) declared no history of alcohol or narcotic abuse, 3) were not taking currently pharmacological treatment for any reason, 4) had no evidence of current adenovirus related or other infections, 5) had any evidence of acute, chronic or metabolic disease other than obesity, 6) were not currently using a specific diet, 7) were not currently involved in any weight control programs and 8) had body weight stable in past 3 months before the study. In addition, pregnant and breastfeeding women were excluded from this study. Before data collection subjects were informed about the aim of the study and signed consent for their participation in the study. The study was approved by the local Bioethics Committee in Collegium Medicum, Bydgoszcz, Poland. According to the World Health Organization criteria these subjects were classified according to determined body-mass index (BMI) value as normal weight (NW, BMI < 25 kg/m^2) and overweight/obese (OW/OB, BMI ≥ 25 kg/m^2) [21-23].

Current BMI of each subject was computed from height which was measured to the nearest 0.5 cm using a height scale and weight which was determined to the nearest 0.1 kg using a digital electronic scale (Radwag, Radom, Poland) with the subjects wearing light indoor clothing and no shoes. Waist and hip circumference of each participant were measured to the nearest 0.1 cm and were used to calculate waist-to-hip ratio (WHR). Three consecutive measurements of four skinfolds thickness (triceps, biceps, subscapular and suprailiac) were performed to the nearest 0.1 mm using electronic skinfold caliper Skyndex I (Caldwell-Justiss & Co., Fayetville, AR, USA). Determined BMI and anthropometric values were used for calculations of percentage of body fat mass (%FM) [22,24]. Total body fat was calculated according to Durnin and Womersley formula [34].

Fasting venous blood was drawn, allowed to clot for 30 min, centrifuged at 3000×*g* for 10 minutes and immediately divided into aliquots. Serum specimens were frozen and stored at -40°C until serological assays were performed. Measurements of serum total cholesterol (TChol, mmol/L) and high density lipoprotein cholesterol (HDLChol, mmol/L) concentration were performed by cholesterol oxidase phenol aminoantipyrine enzymatic assay with spectrophotometric detection [35]. Triglycerides (TG, mmol/L) were determined by glycerol-3-phosphate oxidase phenol aminoantipyrine enzymatic method according to Allain *et al.* [36]. In these both procedures the reagents and automated analyzer BTS 310 from Biosystems (Barcelona, Spain) have been applied. Serum low density lipoprotein cholesterol (LDLChol) was calculated using the Friedewald *et al.* [37] formula: LDLChol = [TChol – (HDLChol – TG/2.2)], and women with TG concentrations greater than 4.5 mmol/L were excluded from the analyses. Detection limit was 18 μmol/L and 8 μmol/L, with linearity up to 6.8 mmol/L and 26.0 mmol/L, in case of, respectively, TG and TChol determination by mentioned enzymatic method. The intra-assay coefficient of variation (CV) characterizing repeatability was < 1.7% and <1.1 %, while the inter-assay CV related with reproducibility was < 2.6 % and < 1.9 % for, respectively, TG and TChol enzymatic analyses.

The qualitative serotype non-specific analyses of antibodies to human adenovirus were performed with use of the commercially available enzyme-linked immunosorbent assay (NovaLisa^{TM}, NovaTec Immundiagnostica GmbH, Dietzenbach, Germany) according to manufacturer's instruction. In brief, serum samples were tested for IgG, IgA and IgM antibodies to human adenovirus on the microtiter strip well coated with human adenovirus antigen to bind corresponding antibodies of specimens. Sera were diluted 1:100 in phosphate buffer and 100 μL was added to each microtiter wells and incubate one hour at 37 °C. After incubation plates were washed three times and 100 μL of horseradish peroxidase labelled anti-human IgG, IgA or IgM conjugate was added. After 30 min at room temperature and three times washing, the immune complex formed by the bound conjugate was visualized by adding tetramethylbenzidine substrate which gives a blue reaction product. After 15 min at room temperature sulphuric acid was added to stop the reaction. Absorbance was read at 450 nm by an ELISA Microwell Plate Reader (Synergy HT, Bio-Tek Instruments Inc., USA). Samples were considered positive if the absorbance value was higher that 10% over the cut-off (*i.e.* 0.38), negative if lower than 10% below the cut-off and grey zone, if absorbance value of 10% above or below the cut-off.

Before statistical analysis, normal distribution of the variances were evaluated using Shapiro-Wilk test. Serum TG, TChol and LDLChol concentrations were given to a log-transformation because of non-normal distribution. The results are presented as mean value ± standard deviation for continuous variables, and as frequency distributions for categorical variables. Differences between the groups were tested by $\chi 2$ for categorical variables and by the Mann Whitney U-test for continuous variables. Multiple regression analysis was performed, including BMI, age and combined seropositivity (IgG^+/IgA^+) both for IgG and IgA antibodies to adenovirus as independent variables and serum TG, TChol and LDLChol concentrations as dependent variables. Additionally, median values for obesity indicators and serum TG, TChol and LDLChol were calculated in whole cohort of females (n = 71) and subjects were divided into those above and below median values. All statistical analyses were undertaken with Statistica PL v. 6.1 software (Stat-Soft, Inc., Tulsa, OK, USA). A value of $p < 0.05$ was considered as statistically significant.

3. RESULTS

Baseline characteristics of the studied population are summarized in Table **1**. The average age of the participants was 45.9 ± 13.6 years. Comparison of the OW/OB females with normal weight (NW) individuals showed a significantly higher BMI, %FM, TChol and TG concentrations in the OW/OB group. The prevalence of adenovirus IgG^+ or both

IgG^+ and IgA^+ antibodies in the whole studied cohort (n = 71) was 90.0 and 18.6%, respectively. Whereas IgM antibodies to adenovirus were not detected in any studied subjects. There were no significant association between adenovirus infection and being overweight or obese (Table **1**). The frequency distributions of seropositivity for IgG and both IgG and IgA antibodies (*i.e.* combined seropositivity (IgG^+/IgA^+)) to adenovirus did not differ between OW/OB and NW subjects (92.0 *vs* 85.0 and 20.0 % *vs* 15.0 %, respectively). The OW/OB subjects with combined serpopositivity both for IgG and IgA antibodies, which suggestive chronic infection, had similar obesity indicators (BMI, %FM and WHR), but significantly lower serum TG, TChol and LDLChol cholesterol concentrations in comparison with seronegative (IgG^-/IgA^-) OW/OB subjects (Table **2**). Similar relationship was observed with all subjects taken together, but there were no associations between obesity indicators BMI, WHR, %FM and determined serum lipids indices with antibodies status in the NW group. It may be explained by the fact that in the NW group were only three subjects with combined seropositivity for both IgG and IgA antibodies to adenovirus (IgG^+/IgA^+). There were no differences in BMI, WHR, %FM and serum lipids between subjects negative (IgG^-) or positive (IgG^+) only for IgG antibodies (data not shown). In addition, it should be noted that subjects positive both for IgG and IgA antibodies to adenovirus (combined IgG^+/IgA^+) had significantly depressed TG, TChol and LDLChol cholesterol in comparison with subjects with only IgG^+ positive antibody status (data not shown). In addition, subjects with TG, TChol and LDLChol cholesterol distribution below the median values of the considered variables had significantly ($p < 0.05$) higher prevalence of combined (double) seropositivity for both IgG and IgA antibodies to adenovirus (IgG^+/IgA^+) than those subjects which were classified to the sub-group with variables above median values (Table **3**).

In the multivariate regression models with TG concentrations as dependent variable and combined seropositivity for both IgG and IgA antibodies to adenovirus (IgG^+/IgA^+), BMI and age of subjects as independent

Table 1. Characteristic of the Study Population (Mean ± SD)

Variables	All Subjects (n = 71)	OW/OB Group (n = 50)	NW Group (n = 21)
Age (years)	45.9 ± 13.6	48.8 ± 12.5	38.7 ± 14.5
BMI (kg/m^2)	29.5 ± 6.0	32.5 ± 4.3*	22.1 ±1.8*
% FM (%)	38.5 ± 6.5	42.0 ± 3.7*	31.8 ± 5.1*
WHR	0.8 ± 0.12	0.82 ± 0.13	0.77 ± 0.06
TChol (mmol/L)	5.16 ± 0.89	5.21 ± 0.93*	5.01 ± 0.79*
HDLChol (mmol/L)	1.65 ± 0.29	1.59 ± 0.27	1.79 ± 0.30
LDLChol (mmol/L)	2.83 ± 0.87	2.91 ± 0.88	2.65 ± 0.83
TG (mmol/L)	1.13 ± 0.37	1.19 ± 0.40*	1.00 ± 0.24*
Adenovirus IgG seropositive (%)	90.0	92.0	85.0
Combined seropositivity (IgG^+/IgA^+) to adenovirus (%)	18.6	20.0	15.0

*Statistically significant differences between OW/OB and NW group ($p < 0.05$).

Table 2. Characteristics of Subjects with Combined Seropositivity (IgG^+/IgA^+) and Combined Seronegativity (IgG^-/IgA^-) for Adenoviruses in Each of Studied Female Groups

Variables	All Subjects (n = 71)		OW/OB Group (n = 50)		NW Group (n = 21)	
	Seropositive IgG^+/IgA^+	Seronegative IgG^-/IgA^-	Seropositive IgG^+/IgA^+	Seronegative IgG^-/IgA^-	Seropositive IgG^+/IgA^+	Seronegative IgG^-/IgA^-
Age (years)	41.8 ± 13.4	47.3 ± 13.4	44.7 ± 12.9	50.5 ± 11.3	32.0 ± 5.0	39.9 ± 15.4
BMI (kg/m^2)	29.6 ± 5.90	29.5 ± 5.20	32.0 ± 4.5	32.6 ± 4.4	21.7 ± 1.5	22.1 ± 1.9
%FM (%)	37.4 ± 6.40	39.7 ± 6.10	40.4 ± 2.6	42.6 ± 3.8	28.4 ± 5.9	32.6 ± 4.8
WHR	0.79 ± 0.06	0.81 ± 0.13	0.81 ± 0.06	0.82 ± 0.15	0.72 ± 0.00	0.78 ± 0.06
TChol (mmol/L)	4.63 ± 0.68[a]	5.27 ± 0.89[a]	4.52 ± 0.57[b]	5.38 ± 0.92[b]	4.99 ± 1.03	5.01 ± 0.78
HDLChol (mmol/L)	1.65 ± 0.29	1.65 ± 0.28	1.68 ± 0.26	1.57 ± 0.27	1.55 ± 0.41	1.85 ± 0.26
LDLChol (mmol/L)	2.43 ± 0.49[a]	2.93 ± 0.591[a]	2.24 ± 0.32[b]	3.07 ± 0.90[b]	2.92 ± 0.58	2.59 ± 0.88
TG (mmol/L)	0.91 ± 0.25[a]	1.19 ± 0.38[a]	0.92 ± 0.26[b]	1.26 ± 0.41[b]	0.87 ± 0.29	1.02 ± 0.24

[a,b]The same letter indicating statistically significant differences ($p < 0.05$) between combined (double) seropositive (IgG^+/IgA^+) and combined (double) seronegative (IgG^-/IgA^-) group.

Table 3. Proportion of Subjects (%) with Combined Seropositivity (IgG^+/IgA^+) to Adenovirus for which Obesity Indicators and Serum Lipids Concentrations have been Classified Below and Above the Median Values in Whole Studied Population of Females (n = 71)

Variables	Below Median (%)	Above Median (%)	*p*
BMI (kg/m^2)	19.4	17.6	0.43
%FM	24.1	16.1	0.43
TChol (mmol/L)	29.4	8.3	**0.02***
HDLChol (mmol/L)	17.2	20.0	0.78
LDLChol (mmol/L)	27.6	10.0	**0.04***
TG (mmol/L)	28.6	8.6	**0.03***

*Statistically significant differences between "Below median" and "Above median" values ($p < 0.05$).

variables, the independent association of serum TG with adenovirus infection ($p < 0.05$) were found, but BMI and age of females did not remain statistically significant factors ($p > 0.05$) (see Table **4**). The same model for serum cholesterol concentrations showed that there were independent associations ($p < 0.05$) between TChol concentrations and combined seropositivity for adenovirus (IgG^+/IgA^+) and age of females in this group. On the contrary, when other variables were controlled no association of adenovirus infection with serum LDLChol cholesterol concentration was found.

4. DISCUSSION

A large of number of adenoviruses circulate freely in environment, so the high seroprevalence of adenovirus antibodies are common worldwide. The frequency distribution of seropositivity for IgG antibodies to adenovirus in the whole group (n = 71) of studied subjects was 90.0 %. This is in agreement with some previous studies reporting a prevalence of 72 - 98.8% of adenovirus neutralizing antibodies in the immunocompetent adult individuals living in The Netherlands [38], Germany [10,38], Gambia [8], South Africa [8], and USA [8,10].

In contrast to the other studies, we could not report higher prevalence of adenovirus seropositivity in the OW/OB group of women in comparison with the NW individuals, even when the result were analyzed in the sub-groups with BMI below and above the median values of studied variables characterizing obesity and serum lipids.. There were also no differences in obesity indicators (BMI, %FM and WHR) between any of adenovirus seropositive and seronegative groups. However, Atkinson *et al*. [17] reported that human adenovirus Ad-36 is associated with development of human obesity. They noted a highly significant differences in body mass index between Ad-36 antibodies positive and negative individuals in the whole population as well as between individuals whether they were obese or non-obese. Whereas, Dhurandhar *et al*. [39], So *et al*. [16], Whigham *et al.* [40], Vangipuram *et al*. [41], Pasarica *et al.* [42], Rogers *et al.* [43], and Bouwman *et al.* [44] in studies with human adenovirus demonstrated that, respectively, Ad-36, Ad-5 and Ad-37 increases adiposity in several animals models independent of food intake. Simultaneously, it was reported that adiposity-promoting effect is specific and not every human adenoviruses are associated with increase of obesity [15,17,40]. After adenoviral infection there are two types antibodies generated: non-specific total antibodies, which are present in individuals who have been exposed to any adenovirus, and serotype-specific neutralizing antibodies generated against adenoviral serotype responsible for the infection [45]. So it is really possible that the lack of associations between adenovirus infections and obesity indicators (BMI, %FM and WHR) observed in our study could be due to the fact that we only investigated the presence of non-specific total adenovirus antibodies.

The major findings of our study are the associations of adenovirus infection with serum TG and TChol concentrations which remained statistically significant ($p < 0.05$) after controlling for BMI and age of subjects. It was also interesting that these associations have been noted only among subjects who were seropositive both for IgG and IgA antibodies to adenovirus (combined seropositivity - marked in text as IgG^+/IgA^+). These findings suggest that only chronic adenovirus infection may be engaged in changes of serum lipid levels in obese group of females. Our results are in accordance with earlier findings of Atkinson *et al.* [17], who reported that levels of TG and TChol were paradoxically significantly lower in Ad-36 antibodies

Table 4. Results of Multiple Regression Model of Selected Markers of Serum Lipid Concentrations

Variables	TG		TChol		LDLChol	
	β – Coefficient	*p*	β – Coefficient	*p*	β – Coefficient	*p*
Age (years)	0.156	0.190	0.297	0.013*	0.235	0.088
BMI (kg/m^2)	0.123	0.300	0.034	0.767	0.049	0.707
Combined seropositivity (IgG^+/IgA^+) to adenovirus (%)	-0.263	0.020*	-0.232	0.044*	-0.166	0.211

*Statistically significant ($p < 0.05$).

positive individuals than in Ad-36 antibodies negative individuals. Similar associations have been observed in animal model by Dhurandhar *et al*. [14,46]. However, in contrast to Ad-36, it has been demonstrated that Ad-37 has a different effect on serum lipids [14]. Ad-37 decreased serum TG similar to Ad-36, but simultaneously caused a significant increase of serum TChol concentration in animal models [46]. These data suggests that, similar to adiposity-promoting effect, changes of serum lipid levels caused by adenovirus infection are different in dependence of adenoviral serotype responsible for the infection. Till today the detailed molecular mechanism of alterations in serum lipids by adenovirus infection have still been uncovered [47]. Atkinson *et al.* [17] postulate that at least two differ mechanisms, confirmed in animal models or *in vitro* studies, *i.e.* Ad-36 related hypothalamus damage or Ad-36 linked altering of adipocyte differentiation, are involved to increase adiposity and serum lipid changes in obese humans with positive antibody status to Ad-36 adenovirus. However, recently the role of upregulated adipogenesis-related genes and activation of peroxisome proliferator-activated receptors PPAR-γ signaling pathway in the Ad-36 induced lipid accumulation by human mesenchymal stem cells have been postulated on the results of microarray assay and gene set enrichment analysis [48]. In addition, the significance of the increased oxidative stress condition in the mechanism of adenovirus infection related obesity have been suggested in the currently published report [49].

It should be noted that there are some limitations of our study including a small sample size of studied groups and restriction participants to females only. The most important limitation was using an non-specific ELISA test which provide a measurement of the total anti-adenovirus antibody level, so the results of our study can not explain which serotype of adenovirus is connected with alternations in serum lipid levels. Whereas, the impact of adenovirus serotype differences in changes of serum lipid levels in obese humans have been reported earlier [17,37,40,48].

In conclusion, our results suggested that infection of some adenoviruses may predispose to altered serum lipids in adult, middle aged, OW/OB female subjects. But further research with use of measurements of the serotype-specific neutralizing antibodies to adenovirus or isolation/identification of specific adenovirus like particles in serum and adipose tissue samples using convective interaction media (CIM) monolithic column technology [50] in this group of OW/OB females should be performed.

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CONFLICT OF INTEREST

There is no conflict of interest.

ABBREVIATIONS

BMI = Body mass index

CIM = Convective interaction media

ELISA = Enzyme linked immunosorbent assay

%FM = Percentage of body mass

HDL = High density lipoproteins

HDLChol = Serum HDL cholesterol

IgG^+/IgA^+ = Combined seropositivity to both IgG and IgA antibodies

LDL = Low density lipoproteins

LDLChol = Serum LDL cholesterol

NW = Normal weight subjects

OW/OB = Overweight/obese subjects

PPAR-γ = Peroxisome proliferator-activated receptors

TChol = Serum total cholesterol concentration

TG = Serum triglyceride concentration

WHR = Waist-to-hip ratio

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