Plasma ghrelin levels in non smokers and passive as well as active smokers: relation to urinary cotinine

T H E S I S

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Abstract

Objective: To evaluate the effect of smoking on plasma ghrelin levels in passive and active smokers and to correlate their levels with body mass index and urinary cotinine.

Patients and Methods: The present study included 85 healthy male subjects, divided into three groups; Group I (n=20) non smokers control group Group II (n=20) passive smokers and Group III (n=45) active smokers.For all subjects, the following investigations were performed; plasma glucose,alanine aminotransferase (ALT), creatinine, urinary cotinine by RIA and plasma ghrelin by ELISA. In addition to history taking and physical examination to exclude any organic disease. Also,determination of body weight and measurement of height were done to calculate body mass index .

Results: The mean plasma ghrelin levels in active smokers were significantly lower vs non smokers and passive smokers ($p<0.001$) while, passive smokers showed no significant difference in ghrelin levels compared to non smokers($p >0.1$).Also, a significant negative correlations were observed between plasma ghrelin levels and body weight ($r = -0.472, p<0.005$), body mass index ($r = -0.798, p<0.001$), systolic blood pressure ($r = -0.671$, $p<0.001$),diastolic blood pressure ($r =-0.562$, $p>0.001$),fasting plasma glucose($r=-0.334$, $p<0.05$), postprandial plasma glucose ($r = -0.396$, $p<0.02$), plasma creatinine ($r = -0.575$, $p<0.001$)and plasma ALT activity ($r=-0.457, p<0.05$).
Conclusion:Cigarette smokers had significantly lower plasma ghrelin level, a mechanism which might have a role in long term regulation of body weight.

Key words: Ghrelin, BMI, Cigarette smoking and Nicotine.

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List of Abbreviations

ACTH	Adrenocorticotrophic hormone
AFP	Alpha fetoprotein
AGRP	Agouti related protein
AIDS	Acquired immune deficiency syndrome
AMPK	Adenosine monophosphate activated kinase
AN	Anorexia nervosa
AN-BP	Anorexia nervosa – binge eating/purging type
AN-R	Anorexia nervosa –restricting type
BMI	Body mass index
BN	Bulimia nervosa
BN-P	Bulimia nervosa –purging type
CHF	Chronic heart failure
c DNA	Complementary deoxy ribonucleic acid
cAMP	Cyclic adenosine mono phosphate
CLD	Chronic liver disease
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CRC	Cancer Research Campaign
CPFs	Cancer potency factors
EC	Enterochromaphin
ED	Erectile dysfunction
ETS	Environmental tobacco smoke
GABA	Gamma amino butyric acid
GFR	Glomerular filtration rate
GH	Growth hormone
GHR	Growth hormone receptors
GHRH	Growth hormone releasing hormone

GHRP-6	Growth hormone releasing peptide-6
GHS	Growth hormone secretagogues
GPCR	G protein coupled receptors
HCC	Hepatocellular carcinoma
HD	Hemodialysis
HDL	High density lipoprotein
HPV	Human papilloma virus
ICAM	Intracellular adhesion molecules
IESR	Institute of Environmental Science and Research
IGF-1	Insulin - like growth factor-1
IL	Interleukin
IQ	Intelligence quotient
IRS-1	Insulin receptor substrate -1
LVEF	Left ventricular ejection fraction
MAO	Mono amine oxidase
mRNA	Messenger ribonucleic acid
NAFLD	Non alcoholic fatty liver disease
NAS	National academy of sciences
NDMA	N-nitrosodimethylamine
NIH	National institute of health
NNN	N- nitrosonornicotine
NP	N- nitrosopyrrolidine
NPY	Neuropeptide Y
OGTT	Oral glucose tolerance test
PAH	Polynuclear aromatic hydrocarbon
PD	Peritoneal dialysis
PEPCK	Phosphoenol pyruvic carboxykinase
PM	Post meridiem

PNS	Peripheral nervous system
PREP	Potential reduce –exposure products
PRL	prolactin
PVN	Paraventricular nuclei
RCC	Renal cell carcinoma
REL	Reference Exposure level
T2DM	Type 2 diabetes mellites
T.I .A	Transient ischemic attacks
TM	Trans membrane
TSH	Thyroid stimulating hormone
USA	United States of America
WHO	World health organization

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Introduction

Tobacco use is the leading preventable cause of death in most countries. Smoking cessation is an important strategy for reducing the morbidity and mortality associated with tobacco-related diseases. An inverse relationship between nicotine use and body weight has been reported, in which body weight tends to be lower among smokers than among nonsmokers. Smoking abstinence results in an increase in body weight for both males and females. Pharmacological treatment for smoking cessation attenuates weight gain. The importance of smoking cessation as a contributing cause of the current obesity epidemic has been little studied. Although the mechanisms are unclear, there is evidence that dopamine and serotonin are appetite suppressants. The administration of nicotine, regardless of the delivery system, acutely raises the levels of these neurotransmitters in the brain, reducing the need for energy intake and consequently suppressing appetite. In addition, nicotine has a direct effect on adipose tissue metabolism, influencing the rate of weight gain following smoking cessation. Leptin, ghrelin and neuropeptide Y are substances that might constitute factors involved in the inverse relationship between nicotine and body mass index ***(Chatkin and Chatkin, 2007).***

Ghrelin is an important regulator of energy balance because it has been demonstrated to increase appetite and food intake and to modulate insulin secretion ***(Ariyasu et al., 2001 and Egido et al., 2002)***. Ghrelin is one of the numerous, recently described, molecules implicated in energy homestasis. It has been shown in rodents that subcutaneous administration of ghrelin causes weight gain through the increase in food

intake and reduction of fat utilization ***(Nakazato et al., 2001).*** Similarly, intravenous ghrelin injection in humans markedly enhances appetite and increases food intake ***(Wren et al., 2001).*** Thus, ghrelin is the first circulating hormone proven to stimulate food intake in man.

Ghrelin, a 28-amino acid peptide with octanoyl-modification at the third serine residue (Ser-3) was purified from stomach extract. Ghrelin is multifunctional peptide implicated in glucose and lipid metabolism, reproduction, gastrointestinal function, cardiovascular function, cellular proliferation, immunomodulation and bone physiology in addition to GH release, and food intake ***(Hosoda et al., 2006 , Kojima and Kangawa, 2005;Soares and Leite-Moreira, 2008).***

The relationships between cigarette smoking and body weight have attracted considerable attention because smokers showed a lower body weight than non-smokers **.** ***Lee et al. (2006)*** and many studies reported that smoking cessation increases body mass index and cause weight gain.

Aim of the Work

The present study aimed to investigate the plasma ghrelin levels in a group of healthy subjects who are active smokers, passive smokers and in a healthy group subjects who are non smokers and not exposed to tobacco smoke. Plasma ghrelin levels of all groups will be correlated to their urinary cotinine and also to their body mass index in order to evaluate the effect of smoking on plasma ghrelin levels which might have a role in the long term regulation of body weight.

Ghrelin

The growing family of synthetic growth hormone (GH) secretagogues (GHSs) (***Camanni et al., 1998)*** consist of peptides and non peptides structurally derived from metenkephalin and synthesized by Bowers and Collaborators in the early 1980 ***(Bowers, 1998 and Momany et al., 1981).*** Since the peptidyl GHS have very low oral bioavailability and short half-life, several small non-peptidyl molecules have been designed which are less susceptible to degradation and have higher bioavailability.The spiroindolin derivative MK-0677 is a small non-peptidyl GHs with excellent oral bioavailability ***(Smith et al., 1997).***

Nomenclature:

The name ghrelin is based on "ghre," a word root in Proto-Indo-European languages for "grow," in reference to its ability to stimulate GH release. Ghrelin is the first known case of a peptide hormone modified by a fatty acid ***(Kojima et al., 1999).***

Structure of Ghrelin:

Ghrelin (lipopeptide) is a 28-residue peptide with an n-octanoyl modification at the hydroxyl group of the 3rd serine, rarely threonine, which is essential for binding to the growth hormone secretagogue receptor la (GHSRA-la), and n-octanoyl bearing ghrelin known as active ghrelin (acylated) ***(Aydin, 2007 and Kojima and Kanagawa, 2005).***De-acylated ghrelin, however, is not totally inactive, has influence on cell

proliferation and adipogenesis (***Aydin, 2007),*** but in term of the "active" form may be more physiologically crucial.

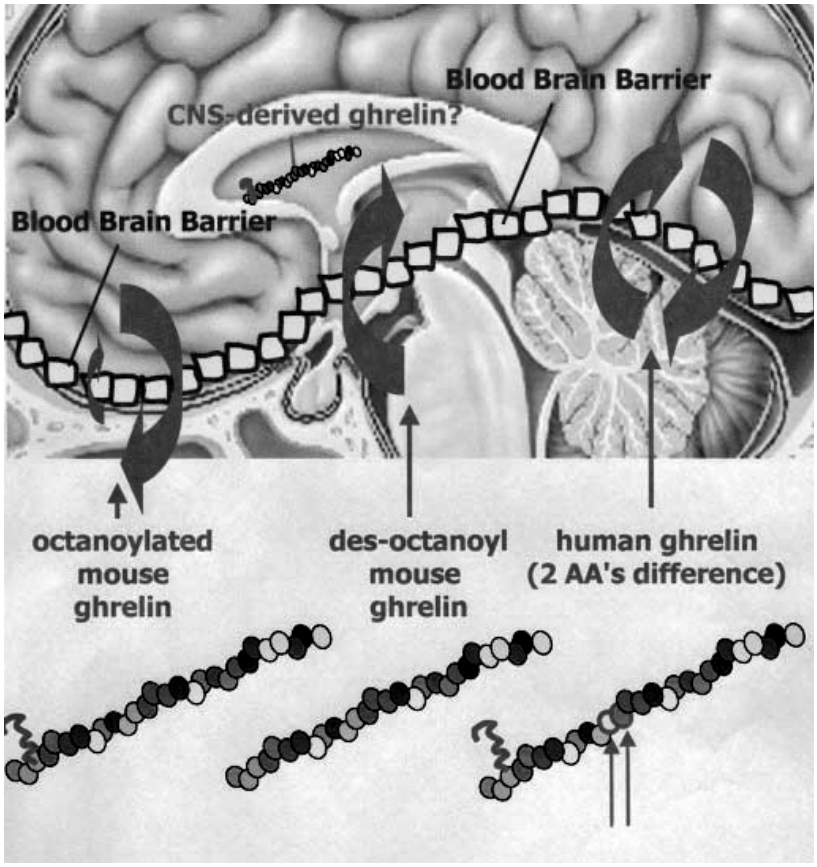

Fig. (1): Differential transport of ghrelin, des-octanoyl mouse gbrelin, and human ghrelin across the blood-brain barrier in mice (William et al., 2002).

Ghrelin was first described in ***1999*** by ***Kojima and Co-workers*** as the endogenous ligand for GHS-R. There is no structural homology between Ghrelin and synthetic ligands GHSs. Ghrelin is a peptide consisting of 28 amino acids and its sequence is highly conserved between various species. All characterized ghrelin derivatives are synthesized from prepro-ghrelin, the same ghrelin precursor of 117 amino acids, through alternative processing. The studies on activity of partially digested ghrelin and its derivatives revealed that the N-terminal portion, consisting of first 4-5 residues, is the active core of the molecule ***(Matsumoto et al.,2001).***Ghrelin bioactivity is ensured by post-translational acylation with octanoic acid at its third serine residue

(Kojima et al., 1999 and Momany et al., 1981), a modification that permits to sustain ghrelin activity ***(Matsumoto et al., 2001 and Hosoda et al., 2003).***

In rat stomach, a second type of ghrelin peptide has been purified and identified as des-Gln14 ***(Hosoda et al., 2000a),*** ghrelin is identical to des-Gln14-ghrelin except for the deletion of Gln14, even retaining the *n*-octanoic acid modification.Des-Gln14-ghrelin has the same potency of activities as that of ghrelin.

Thus two types of active ghrelin peptide are produced in rat stomach: ghrelin and des-Gln14-ghrelin. However, des-Gln14-ghrelin is only present in low amounts in the stomach, indicating that ghrelin is the major active form. In addition, n-decenoyl (C10:1)-modified ghrelin exists in the stomach in small amounts.

Ghrelin, a stomach derived peptide, is the only known circulating orexigenic hormone. It is acylated with a medium-chain fatty acid by the enzyme ghrelin O-acetyltransferase (GOAT), and displays a broad range of activity, from central control of food intake to peripheral functions such as gastric emptying and insulin secretion ***(Kirchner et al.,2010).***

In the course of purifying human ghrelin from the stomach,several minor forms of the peptide were isolated ***(Hosoda et al., 2003)***. These could be classified into four groups by the type of acylation observed at Ser3: nonacylated, octanoylated (C8:0),decanoylated (C10:0), and possibly decenoylated (C10:1). All peptides found were either 27 or 28 amino acids in length, the former lacking the COOH-terminal Arg28, and are derived from the same ghrelin precursor through two alternative pathways. As was the case in the rat, the major active form of human ghrelin is a 28-amino acid peptide with octanoylated Ser3. Synthetic octanoylated and decanoylated ghrelins stimulate the increase of