Salivary Cortisol Can Reflect Adiposity and Insulin Sensitivity in Type 2 Diabetes

Yoko Matsuzawa[1,2], Kenichi Sakurai[1], Jun Saito[2],
Masao Omura[2] and Tetsuo Nishikawa[2]
[1]Clinical Cell Biology and Medicine, Graduate School of Medicine, Chiba University,
[2]Division of Endocrinology and Metabolism, Endocrinology and Diabetes Center,
Department of Medicine, Yokohama Rosai Hospital,
Japan

1. Introduction

Glucocorticoids are well known to play an important role in the regulation of most essential physiological processes (Atanasov & Odermatt,2007). Patients with Cushing`s syndrome show central obesity with insulin resistance, caused by hypersecretion of cortisol (F) (Arnaldi et al.,2004). Obese patients with type 2 diabetes often have symptoms usually observed in patients with Cushing`s syndrome, and F levels might reflect the severity of complications and metabolic abnormalities in diabetes (Chiodini et al.,2007). High levels of F are associated with activation or dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and increased volume of the adrenal glands (Pasquali et al.,2006, Godoy-Matos et al.,2006).

Moreover, various metabolic abnormalities induced by enhanced glucocorticoid activity were found to be not only due to accelerated function of the HPA axis, but also by an impairment in 11β-hydroxysteroid dehydrogenase (11β-HSD) enzymes within the target cells (Godoy-Matos et at.,2006). 11β-HSD has two isoforms: 11β-HSD type 1 (11β-HSD1) mainly works as a reductase which converts inactive cortisone (E) to active cortisol (F) in F target tissues (Walker & Andrew,2006). 11β-HSD type 2 (11β-HSD2) is expressed in mineralocorticoid target tissues such as the distal nephron, colon, and salivary glands (Tannin et al.,1991, Draper & Stewart, 2005, Edwards et al., 1988), converting F to E to protect mineralocorticoid receptors from activation by F.

Animal models demonstrated that activation of 11β-HSD1 exhibited features of metabolic syndrome (Masuzaki et al.,2004, Morton et al.,2004). It was also reported that 11β-HSD1 is increased in subcutaneous adipose tissue in obese patients (Rask et al.,2002, Paulmyer-Lacroi et al.,2002), and higher 11β-HSD1 activity in adipose tissue is associated with features of metabolic syndrome in Caucasians and Pima Indians (Lindsay et al.,2003). Thus, it is suggested that F may play a crucial role in the regulation of adiposity in type 2 diabetes with obesity. On the other hand, the exact role of abnormal glucocorticoid metabolism in the pathogenesis of obesity has not fully been clarified yet. Salivary cortisol has been reported to be in closer agreement with the real adrenocortical function than serum cortisol concentration (Bolufer et al.,1989). Measurement of salivary cortisol was also reported to have several advantages, such as directly reflecting free cortisol level (Vining et al.,1983), and non-invasiveness for sampling (Chen et al.,1985). We, therefore, analyzed samples from

serum, saliva, and 24h-collected urine from obese type 2 diabetic patients as well as healthy subjects in order to evaluate clinical usefulness of salivary cortisol accurately measured by liquid mass spectroscopy in obese patients with type 2 diabetes.

2. Subjects and methods

2.1 Subjects

Eighteen Japanese men without underlying diseases cooperated as the healthy subject group. As the patient group, 23 Japanese male patients with type 2 diabetes, admitted to the Department of Endocrinology and Metabolism in Yokohama Rosai Hospital between March 2006 and March 2007, who met the following conditions were selected:1) waist circumference of 85 cm or greater, 2) stage 1 or 2 diabetic nephropathy, 3) not treated with oral biguanide or thiazolidine derivatives, and 4)understanding the objective of this study and giving written consent. This study was approved by the research ethics committee of Yokohama Rosai Hospital.

2.2 Measurement of serum and salivary steroids

Five ml of blood and 1 ml of saliva were collected before breakfast and supper on the same day under regular conditions of daily life from the controls and patients. Furthermore, 5ml of blood and 1ml of saliva 2 hours after breakfast, lunch and supper, and 20 ml of 24-h accumulated urine were collected on the same day during hospitalization on 7 days after admission from the patient group. The diet consisted of 25kcal/kg for ideal body weight distributed in three meals.

Blood samples were immediately centrifuged, and the sera were stored at -30°C until measurement. Saliva and accumulated urine samples were stored with no processing at -30°C. Cortisol and cortisone in the samples were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Teikoku Hormone Mfg.-Asuka Pharmaceutical Co., Tokyo, Japan).

2.3 Glucose clamp

Euglycemic-hyperinsulinemic glucose clamping was performed in 14 of the patient group using an artificial pancreas (STG-22, Nikkiso, Tokyo, Japan), following the method described previously (Bergman et al.,1985, Nishikawa et al.,1996). Average age, HbA1c and BMI of these patients were not significantly different from those of the whole cases in

	Diabetic subjects	Healthy Volunteers	P value
n	23	18	
Age (yr)	54.7±13.6	48.3±14.3	0.32
BMI(kg/m^2)	28.2± 5.4	22.1±2.0	<.001
Waist circumference（cm）	96.6±12.4	80.8±5.6	<.001
Duration of diabetes (yr)	7.0±4.7	—	—
HbA1c（%）	10.6±1.9	—	—

(data expressed as mean ±SD)

Table 1. Anthropometric measures and background of the subjects

patient group. In patients under drug treatment for diabetes, administration of sulfonylurea and a long-acting insulin preparation was suspended from the previous evening to avoid their influence on the test.

2.4 Statistical analysis

The subjects' backgrounds are presented as the means ± standard deviation, and the t-test was used to compare healthy subjects with patients. To analyze the correlation between 2 variables, Spearman's correlation coefficient was used, and a level of less than 5% was regarded as significant.

3. Results

3.1 Subject characteristics

The characteristics of the healthy and patient group, including age and BMI, are described in Table 1. The mean duration of illness was 7.0 ± 4.7 years in the patient group, and 14 (60.9%) and 8 (34.8%) patients were under treatment with insulin and oral drugs, respectively at the time of sample collection.
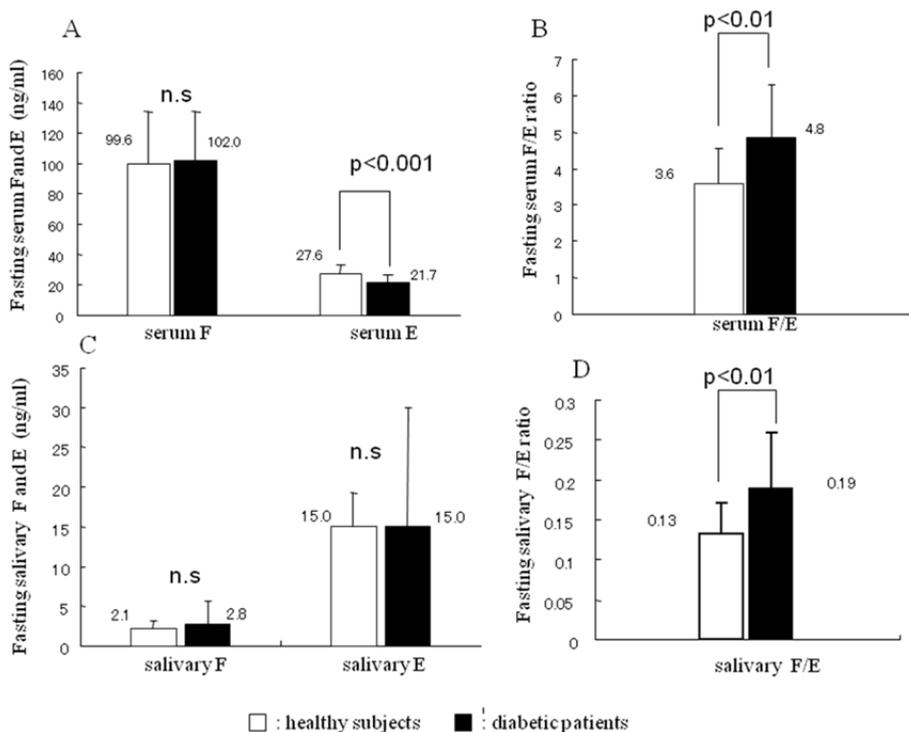
Serum levels of F and E were not different between healthy subjects and diabetic patients (A), and the ratio of F to E was significantly higher in the patient group (B). A similar result was observed for saliva (C, D). Data are expressed as the means±S.D. Statistical significance between healthy and diabetic subjects is described inside the figure.

Fig. 1. Comparison of F, E, and F/E ratios between healthy subjects and diabetic patients in the fasting phase in the morning.

3.2 Relationship between F/E ratio and body weight

As shown in Fig. 1, no significant difference was noted in the blood cortisol (F) level before breakfast between the healthy and patient group, but the cortisone (E) level was lower in the patient group. Accordingly, the ratio of F to E (F/E) in blood was significantly higher in the patient group (3.6 vs. 4.8, respectively, p<0.01).

There was no significant difference in F or E level in saliva before breakfast between the healthy and patient group. Unlike the blood levels, E level was higher than F level in saliva. This is considered to be the influence of 11β-HSD2 expressed in the salivary gland (Tannin et al.,1991). However, the F/E ratio in saliva was significantly higher in the patient group, as in blood (0.13 vs. 0.19, respectively, p<0.01).

There was no correlation between the blood F/E ratio before breakfast and body mass index (BMI) in healthy subjects (Fig.2A), while there was a strong positive correlation between the blood F/E ratio and BMI in the patient group (Fig.2B). This correlation also remained significant after correction with age, HbA1c and blood glucose level before breakfast. Furthermore, a similar result was observed in salivary samples. The salivary F/E ratio tended to show a positive correlation with BMI in diabetic patients, but not in healthy subjects (Fig.2C, D).
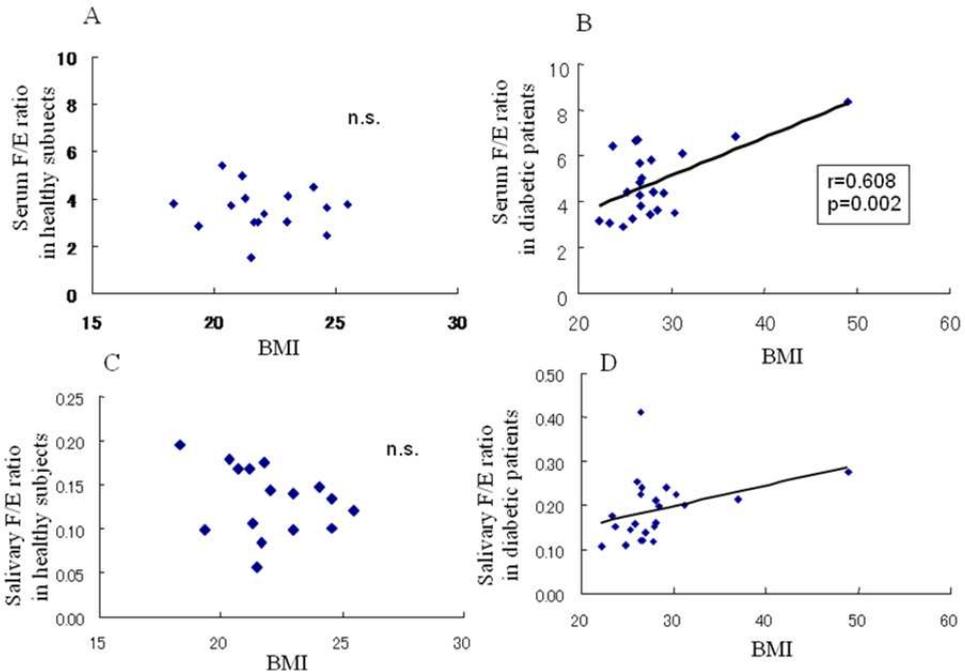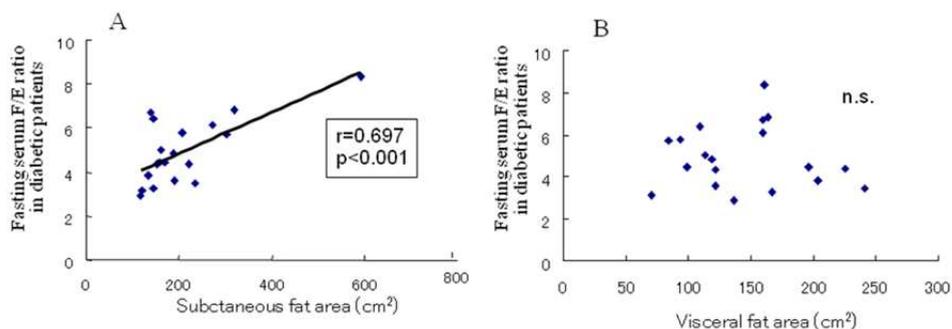

F/E ratio in blood was positively correlated with BMI only in diabetic patients (A, B). F/E ratio from salivary samples also showed a similar tendency, although the correlation was not statistically significant in diabetic patients (C, D).

Fig. 2. Correlation between fasting F/E ratio and BMI.

Fig. 3 shows the relationship between the fasting blood F/E ratio and fat volume assessed by abdominal CT in the patient group. The F/E ratio was positively correlated with the

subcutaneous fat area, but was not correlated with the area of visceral fat. Furthermore, the fasting blood and salivary F/E ratio was strongly correlated with serum leptin level (r=0.652, p<.0.01 for blood, r=0.469, p<.0.05 for saliva), but was not correlated with the plasma concentration of high molecular weight adiponectin or the severity of insulin resistance measured by glucose clamping (data not shown).


Fasting blood F/E ratio positively correlated with subcutaneous fat area (A), but no correlation was found between F/E ratio and visceral fat area (B).

Fig. 3. Relationship between fasting blood F/E ratio and body fat area assessed by CT scan.

### 3.3 Relationship between salivary cortisol and insulin sensitivity
Blood and saliva were collected at 4-time points: before breakfast and 2 hours after each meal in the patient group. Fig. 4 shows the diurnal variation of F after assessing the level before breakfast as 1.0. The F level significantly decreased with time and the variation was larger in saliva than in blood. The within-day variation of the F/E ratio was smaller than that of the F level.
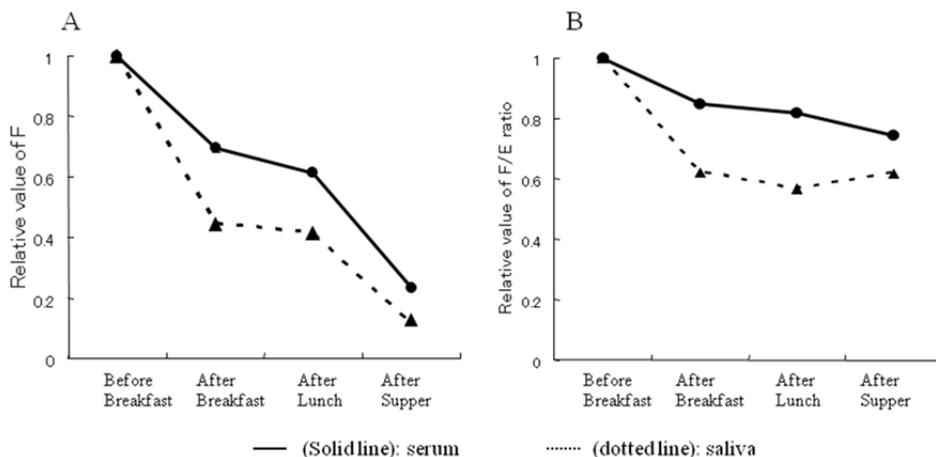
Solid line represents blood samples, and dotted line represents salivary samples. Both blood and saliva had a circadian rhythm in the patients, but salivary F fluctuated more dynamically than blood cortisol within a day.

Fig. 4. Circadian rhythm of F (A) and F/E ratio (B) in diabetic patients.

The salivary F level after breakfast was strongly correlated with the severity of insulin resistance measured by the glucose clamp (Fig. 5). This relationship with insulin resistance was not significantly noted in blood F.
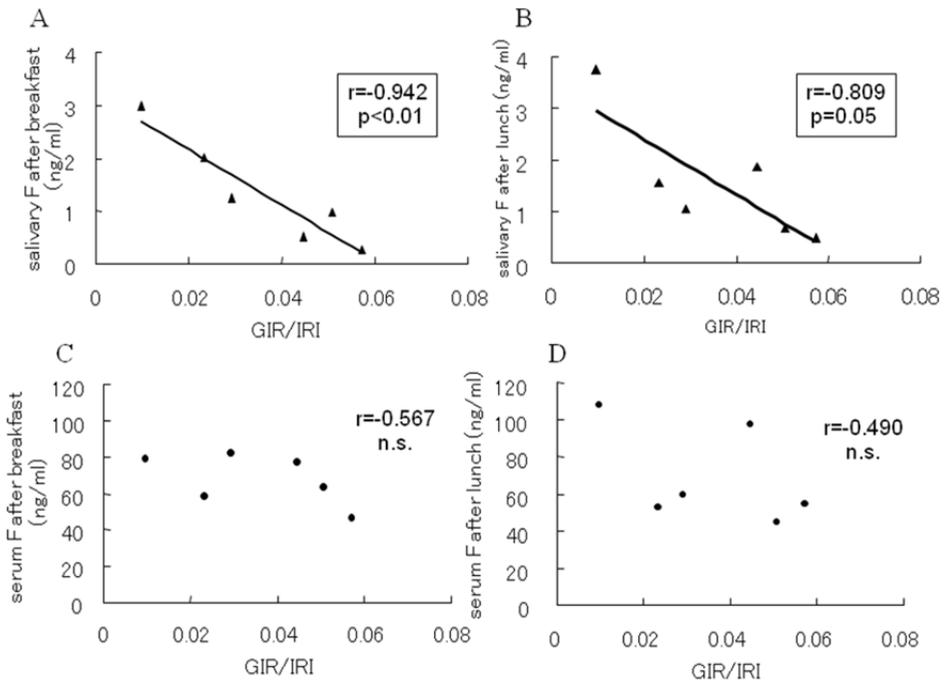
Salivary F after breakfast showed the strongest negative correlation with GIR/IRI (A, B), while serum F(C, D) does not show significant correlation with insulin sensitivity.

Fig. 5. Relationship between insulin sensitivity (GIR/IRI) and F.

4. Discussion

The present study clearly demonstrated that obese patients with type 2 diabetes had a higher fasting F/E ratio in blood and saliva in the morning, comparing with healthy control subjects. Consistent with our results, an elevated blood F/E ratio in patients with type 2 diabetes has been reported (Valsamakis et al.,2004, Homma et al.,2001, Sinha & Caro 1998). Moreover, this is the first report demonstrating a significant increase in the F/E ratio in obese diabetic patients in saliva as well as in blood. Thus, it is suggested that overweight seems to induce much more F formation rather than E production via some mechanism(s) of changing steroidogenic enzymes, including 11β-HSD1 and 2.

Our data also showed a positive relationship between the fasting F/E ratio and BMI, between the fasting F/E ratio and total fat volume, and between the fasting F/E ratio and leptin, in diabetic obese patients. It is, therefore, suggested that the fasting F/E ratio may reflect the severity of adiposity accumulated to both of subcutaneous and visceral areas in obese diabetics, since circulating leptin levels are reported to be the best predictor of total body fat mass (Sinha & Caro,1998). Thus it can be postulated that increased fat mass may

lead to increased F production, at least partly mediated by 11β-HSD1 in adipose tissue, resulting in an elevated F/E ratio after overnight fasting. It was also reported that the expression of 11β-HSD1 is increased in adipose tissue in simple obesity (Rask et al.,2002, Paulmyer-Lacroi et al.,2002, Lindsay et al.,2003), suggesting that the ratio of F to E is a biomarker for assessing the adiposity in obese patients.

Oltmanns et al. (Oltmanns et al.,2006) recently reported that the level of salivary F of which sample was taken between breakfast and lunch was significantly related to metabolic findings in type 2 diabetes, such as fasting and postprandial blood glucose, urinary glucose, and glycosylated hemoglobin, although insulin sensitivity was not directly assessed in their study. Our data demonstrated that a circadian rhythm of F was apparently observed both in blood and saliva, and salivary F 2 hours after breakfast was shown to significantly correlate with insulin sensitivity assessed by euglycemic glucose clamping in obese patients with type 2 diabetes. Salivary F has been reported to have a circadian rhythm, highest in the morning, with lunch followed by a peak (Rosmond et al.,1998), suggesting that the salivary level of F usually decreases before lunch under normal conditions. Thus, it is suggested from our data that the level of salivary F before lunch, reflecting insulin sensitivity, may be up-regulated by food intake after breakfast despite of decreasing ACTH level by diurnal rhythm, since eating was reported to stimulate F secretion and women with abdominal obesity have also been reported to have a greater rise of F in response to food than those with peripheral obesity in simple obesity (Pasquali et al.,1998, Duclos et al.,2005, Korbonits et al.,1996). It had been recently reported that U-shaped associations were apparent between diurnal slope in salivary F and both BMI and waist circumference (Kumari et al.,2010), and also that 6 wk of supplementation with fish oil significantly increased lean mass and decreased fat mass, which were significantly correlated with a reduction in salivary F following fish oil treatment (Noreen et al.,2010).

Moreover, we should consider why salivary F could reflect insulin resistance in diabetic patients, while blood F could not. First, F measured in blood was the total of the free form and protein-bound form, while F in saliva reflected the biologically active blood unbound F level (Vining et al.,1983). The level of blood F can be altered by the concentration of blood F binding globulin (CBG), and CBG levels are shown to correlate negatively with BMI, waist-to-hip ratio, and HOMA (Fernandez-Real et al.,2002). Hence, salivary F is supposed to indicate the level of 'real' F activity in vivo, and thus correlates strongly with insulin resistance. Second, as shown in Fig. 4, salivary F fluctuates more dynamically than blood F within a day. None of our patients were diagnosed as overt or subclinical Cushing syndrome, and their levels of F were within the normal range. However, even a small increase in blood F within the normal range may contribute to abnormal glucose metabolism in metabolic syndrome and type 2 diabetes (Khani & Tayek,2001), and salivary F seems to be more sensitive than blood F to detect subtle changes in the metabolic state.

On the other hand, our results demonstrated that there is not significantly positive correlation between salivary F after breakfast and visceral fat mass. Visceral fat volume is known to be one of the most important factors to determine insulin resistance (Bergman et al.,2007), but our data suggest that F has a significant influence on insulin resistance, and intimately related to total fat volume. Furthermore, many factors including levels of glucose and insulin are supposed to modulate F metabolism after meals. Therefore, measuring salivary F after breakfast in obese diabetic patients may be a useful and noninvasive simple method to predict overall insulin sensitivity and severity of adiposity in such patients.

In conclusion, we tried to investigate the role of blood and salivary F and E in the regulatory mechanisms of obesity in obese men with type 2 diabetes. They had a higher fasting F/E ratio in both blood and saliva than that in healthy controls. Moreover, the fasting F/E ratio showed a significant correlation with total fat volume, suggesting that the fasting ratio of F to E in saliva and blood seems to directly reflect the adiposity in type 2 diabetic patients with obesity. Our data also demonstrated that salivary F after breakfast is suggested to be one of the most useful markers of insulin sensitivity in these patients. On the other hand, our study was conducted with a cross-sectional design, which does not allow us to assume any direct causality of F to insulin resistance. The role of F metabolism in adipogenesis and insulin resistance in type 2 diabetes will require further investigation with a prospective design.

5. Acknowledgment

6. References

Arnaldi G., Mancini T., Polenta B. & Boscaro M. (2004) Cardiovascular risk in Cushing's syndrome. *Pituitary* 7:253-6.

Atanasov A.G., Odermatt A. 2007 Readjusting the glucocorticoid balance: an opportunity for modulators of 11β-hydroxysteroid dehydrogenase type 1 activity? *Endocr Metab Immune Disord Drug Targets*.7:125-40.

Bergman R.N., Finegood D.T. & Ader M. (1985) Assessment of insulin sensitivity in vivo. *Endocr Rev.* 6:45-86.

Bergman R.N., Kim S.P., Hsu I.R., Catalano K.J., Chiu J.D., Kabir M., Richey J.M. & Ader M. (2007) Abdominal obesity: role in the pathophysiology of metabolic disease and cardiovascular risk. *Am J Med* 120: S3-8; discussion S29-32

Bolufer P., Gandia A & Antonio P. (1989) Salivary corticosteroids in the study of adrenal function. *Clin Chem Acta*, 183: 217-225

Chen Y.M, Cintron N.M & Whitson P.A. (1985) Long-term storage of salivary cortisol samples at room temperature. *Clin Chem* 38: 304

Chiodini I, Adda G, Scillitani A, Coletti F, Morelli V, Di Lembo S, Epaminonda P, Masserini B, Beck-Peccoz P, Orsi E, Ambrosi B & Arosio M (2007) Cortisol secretion in patients with type 2 diabetes. *Diabetes Care* 30: 83-88

Draper N & Stewart P.M. (2005) 11β-hydroxysteroid dehydrogenase and the pre-receptor regulation of corticosteroid hormone action. *J Endocrinol* 186: 251-271

Duclos M. Pareira P.M., Barat P., Gatta B. & Roger P. (2005) Increased cortisol bioavailability, abdominal obesity, and the metabolic syndrome in obese women. *Obesity Research* 13: 1157-1166

Edwards CR, Stewart PM, Burt D, Brett L, McIntyre MA, Sutanto WS, de Kloet ER & Monder C. (1988) Localization of 11β-hydroxysteroid dehydrogenase: tissue specific protector of the mineralocorticoid receptor. *Lancet* 2(8618): 986-989

Fernandez-Real JM, Pugeat M, Grasa M, Broch M, Vendrell J, Brun J & Ricart W. (2002) Serum corticosteroid-binding globulin concentration and insulin resistance syndrome: a population study. *J Clin Endocrinol Metab* 87: 4686-90

Godoy-Matos A.F., Vieira A.R & Meirelles R.M. (2006) The potential role of increased adrenal volume in the pathophysiology of obesity-related type 2 diabetes. *J Endocrinol Invest* 29:159-63

Homma M, Tanaka A, Hino K, Takamura H, Hirano T, Oka K, Kanazawa M, Miwa T, Notoya Y, Niitsuma T & Hayashi T. (2001) Assessing systemic 11β-hydroxysteroid dehydrogenase with serum cortisone/cortisol ratios in healthy subjects and patients with diabetes mellitus and chronic renal failure. *Metabolism* 50: 801-804

Khani S, Tayek J.A. 2001 Cortisol increases gluconeogenesis in humans: its role in the metabolic syndrome. *Clinical Science* 101: 739-747

Korbonits M, Trainer PJ, Nelson ML, Howse I, Kopelman PG, Besser GM, Grossman AB & Svec F. (1996) Differential stimulation of cortisol and dehydroepiandrosterone levels by food in obese and normal subjects: relation to body fat distribution. *Clin Endocrinol*. 45: 699-706.

Kumari M, Chandola T, Brunner E & Kivimaki M. (2010) A nonlinear relationship of generalized and central obesity with diurnal cortisol secretion in the Whitehall II study. *J Clin Endocrinol Metab*. 95:4415-23.

Lindsay R.S., Wake D.J., Nair S., Bunt J., Livingstone D.E.W., Permana P.A., Tataranni P.A. & Walke B.R. (2003) Subcutaneous adipose 11β-hydroxysteroid dehydrogenase type 1 activity and messenger ribonucleic acid levels are associated with adiposity and insulinemia in Pima Indians and Caucasians. *J Clin Endocrinol Metab* 87: 2701-2705

Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR & Flier JS. (2004) A transgenic model of visceral obesity and the metabolic syndrome. *Science* 294: 2166-2170

Morton NM, Paterson JM, Masuzaki H, Holmes MC, Staels B, Fievet C, Walker BR, Flier JS, Mullins JJ & Seckl JR. (2004) Novel adipose tissue-mediated resistance to diet-induced visceral obesity in 11β-hydroxysteroid dehydrogenase type 1 deficient mice. *Diabetes* 53: 931-938

Nishikawa T, Iizuka T, Omura M, Kuramoto N, Miki T, Ito H & Chiba S. (1996) Effect of mazindol on body weight and insulin sensitivity in severely obese patients after a very-low-calorie diet therapy. *Endocr J*. 43:671-7.

Noreen EE, Sass MJ, Crowe ML, Pabon VA, Brandauer J & Averill LK. (2010) Effects of supplemental fish oil on resting metabolic rate, body composition, and salivary cortisol in healthy adults *Journal of the International Society of Sports Nutrition* 2010, 7:31

Oltmanns KM, Dodt B, Schultes B, Raspe HH, Schweiger U, Born J, Fehm HL & Peters A. (2006) Cortisol correlates with metabolic disturbances in a population study of type 2 diabetic patients. *Eur J Endocrinol* 154: 325-311

Pasquali R, Biscotti D, Spinucci G, Vicennati V, Genazzani AD, Sgarbi L & Casimirri F. (1998) Pulsatile secretion of ACTH and cortisol in premenopausal women: effect of obesity and body fat distribution. *Clin Endocrinol*.48: 603-12,

Pasquali R, Vicennati V, Cacciari M & Pagotto U. (2006) The hypothalamic-pituitary-adrenal axis activity in obesity and the metabolic syndrome. *Ann N Y Acad Sci*.1083:111-28.

Paulmyer-Lacroi O, Boullu S, Oliver C, Alessi MC & Grino M (2002) Expression of the mRNA coding for 11β-hydroxysteroid dehydrogenase type 1 in adipose tissue from obese patients: an *in situ* hybridization study. *J Clin Endocrinol Metab* 87: 2701-2705

Rask E., Olsson T., Soderberg S., Andrew R., Livingstone D.E., Johnson O. &Walker B.R. (2001) Tissue-specific disregulation of cortisol metabolism in human obesity. *J Clin Endocrinol Metab* 86: 1418-1421

Rask E, Walker BR, Söderberg S, Livingstone DE Eliasson M, Johnson O, Andrew R & Olsson T. (2002) Tissue-specific changes in peripheral cortisol metabolism in obese women: Increased adipose 11β-hydroxysteroid dehydrogenage type 1 activity. *J Clin Endocrinol Metab* 87: 3330-3336

Rosmond R., Dallman M.F. & Bjorntorp P. (1998) Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. *J Clin Endocrinol Metab* 83: 1853-1859

Sinha, M.K. & Caro, J.F. (1998) Clinical aspects of leptin. *Vitam Horm*. 54: 1-30

Stewart PM, Boulton A, Kumar S, Clark PM & Shackleton CH. (1999) Cortisol metabolism in human obesity: impaired cortisone-cortisol conversion in subjects with central adiposity. *J Clin Endocrinol Metab* 84: 1022-1027

Tannin G.M, Agarwal A.K., Monder C, New M.I. & White, P.C. (1991) The human gene for 11β-hydroxysteroid dehydrogenase. Structure, tissue distribution, and chromosomal localization. *J Biol Chem* 266: 16653-16658

Valsamakis G, Anwar A, Tomlinson JW, Shackleton CH, McTernan PG, Chetty R, Wood PJ, Banerjee AK, Holder G, Barnett AH, Stewart PM & Kumar S. (2004) 11β-hydroxysteroid dehydrogenase type 1 activity in lean and obese males with type 2 diabetes mellitus. *J Clin Endocrinol Metab*. 89:4755-61

Vining RF, McGinley RA, Maksvytis JJ & Ho KY. (1983) Salivary cortisol: a better measure of adrenal cortical function than serum cortisol. *Ann Clin Biochem* 20:329-335

Walker B.R. & Andrew R. (2006) Tissue production of cortisol by 11β-hydroxysteroid dehydrogenase type 1 and metabolic disease. *Ann.N.Y.Acad.Science* 1083:165-184
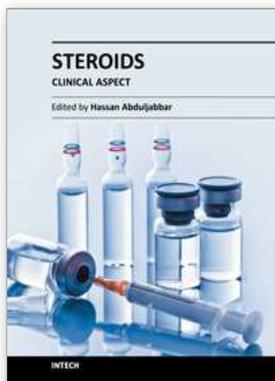
Steroids - Clinical Aspect

Edited by Prof. Hassan Abduljabbar

Steroids: The basic science and clinical aspects covers the modern understanding and clinical use of steroids. The history of steroids is richly immersed and runs long and deep. The modern history of steroids started in the early 20th century, but its use has been traced back to ancient Greece. We start by describing the basic science of steroids. We then describe different clinical situations where steroids play an important role. We hope that this book will contribute further to the literature available about steroids and enables the reader to further understand this interesting and rapidly evolving science.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Yoko Matsuzawa, Kenichi Sakurai, Jun Saito, Masao Omura and Tetsuo Nishikawa (2011). Salivary Cortisol Can Reflect Adiposity and Insulin Sensitivity in Type 2 Diabetes, Steroids - Clinical Aspect, Prof. Hassan Abduljabbar (Ed.), ISBN: 978-953-307-705-5, InTech, Available from: http://www.intechopen.com/books/steroids-clinical-aspect/salivary-cortisol-can-reflect-adiposity-and-insulin-sensitivity-in-type-2-diabetes

INTECH
open science | open minds