WILEY-BLACKWELL

Asian J Androl 2008; 10 (5): 799–807
DOI: 10.1111/j.1745-7262.2008.00421.x

AJA

·*Original Article*·

Insulin and leptin enhance human sperm motility, acrosome reaction and nitric oxide production

Fanuel Lampiao, Stefan S. du Plessis

Division of Medical Physiology, Department of Biomedical Sciences, University of Stellenbosch, Tygerberg 7505, South Africa

Abstract

Aim: To investigate the *in vitro* effects of insulin and leptin on human sperm motility, viability, acrosome reaction and nitric oxide (NO) production. **Methods:** Washed human spermatozoa from normozoospermic donors were treated with insulin (10 μIU) and leptin (10 nmol). Insulin and leptin effects were blocked by inhibition of their intracellular effector, phosphotidylinositol 3-kinase (PI3K), by wortmannin (10 μmol) 30 min prior to insulin and leptin being given. Computer-assisted semen analysis was used to assess motility after 1, 2 and 3 h of incubation. Viability was assessed by fluorescence-activated cell sorting using propidium iodide as a fluorescent probe. Acrosome-reacted cells were observed under a fluorescent microscope using fluorescein-isothiocyanate–*Pisum sativum* agglutinin as a probe. NO was measured after treating the sperm with 4,5-diaminofluorescein-2/diacetate (DAF-2/DA) and analyzed by fluorescence-activated cell sorting. **Results**: Insulin and leptin significantly increased total motility, progressive motility and acrosome reaction, as well as NO production. **Conclusion:** This study showed the *in vitro* beneficial effects of insulin and leptin on human sperm function. These hormones could play a role in enhancing the fertilization capacity of human spermatozoa. *(Asian J Androl 2008 Sep; 10: 799–807)*

Keywords: insulin; leptin; spermatozoa; nitric oxide; motility; acrosome reaction

1 Introduction

The discovery that human ejaculated spermatozoa secrete insulin [1] and leptin [2] has opened a new field of study in reproductive biology. Leptin, a hormone secreted mainly by adipose tissue [3] is known as a regulator of food intake and energy expenditure [4]. It also fulfils many other functions, such as the regulation of neuroendocrine systems, hematopoieses, angiogenesis, puberty and reproduction [5–8]. Studies have shown the presence of leptin receptors on human spermatozoa as well as soluble leptin receptors in seminal plasma [9].

Insulin is mainly produced by the β cells of the pancreas and is important for the promotion of growth, differentiation, and metabolism in somatic cells [10]. It has also been shown to play a role in the regulation of gonadal function [11].

In other cell types, leptin and insulin play a central role in regulation of energy homeostasis, acting on the phosphotidylinositol 3-kinase (PI3K)/protein kinase B pathway that mediates their metabolic effects [12].

Correspondence to: Mr Fanuel Lampiao, Department of Biomedical Sciences, Division of Medical Physiology, University of Stellenbosch, Tygerberg 7505, South Africa.
Tel: +27-21-938-9384 Fax: +27-21-938-9476
E-mail: fannuel@sun.ac.za
Received 2008-02-07 Accepted 2008-04-20

© 2008, Asian Journal of Andrology, SIMM and SJTU. All rights reserved.

Similarly, in uncapacitated sperm, both insulin and leptin increased PI3K activity as well as AktS473 and GSK-3S9 phosphorylation [1, 2], thereby possibly modulating the availability of the spermatozoa's energetic substrates during capacitation. However, the significance of these hormones in male fertility is not properly elucidated.

Recent studies have confirmed the role of nitric oxide (NO) in modulating sexual and reproductive function [13]. The production of NO is catalysed by a family of NO synthase (NOS) enzymes [14]. NOS is responsible for the conversion of *L*-arginine to NO and *L*-citrulline [15] and has been shown to be expressed in spermatozoa [16]. The ability of human spermatozoa to synthesize NO has been shown indirectly by measuring nitrite accumulation [16], as well as *L*-[^{3}H]citrulline generation [17] or directly by means of an isolated NO meter with sensor [18] and flow cytometry [19].

The aim of this study was to investigate the *in vitro* effects of leptin and insulin on human sperm motility, viability, acrosome reaction, and NO production.

2 Materials and methods

2.1 Chemicals

Wortmannin, Ham's F10, leptin, *N*-nitro-*L*-arginine methyl ester (*L*-NAME), propidium iodide (PI), fluorescein isothiocyanate–*Pisum sativum* agglutinin, and progesterone were obtained from Sigma Chemical (St. Louis, MO, USA). Human insulin was purchased from Lilly France (Fegersheim, France). 4,5-Diaminofluorescein-2/diacetate (DAF-2/DA) was from Calbiochem (San Diego, CA, USA).

2.2 Preparation of sperm samples

The 25 donors recruited in this study provided informed consent for a research protocol approved by the University of Stellenbosch Ethics Committee (Tygerberg, South Africa). Fresh semen samples were obtained by masturbation from healthy volunteers after a minimum of 2 days of sexual abstinence according to World Health Organization guidelines [20]. Samples were left to liquefy for 30 min before processing. Motile sperm fractions were retrieved from the samples using a double wash (400 × *g*, 5 min) swim-up technique in Hams medium containing 3% bovine serum albumin (37ºC, 5% CO_2). After 1 h, the supernatant containing motile sperm was collected and divided into aliquots (5 × 10^6/mL).

2.3 Experimental procedure

Insulin and leptin effects were blocked by inhibition of their intracellular effector, PI3K, by wortmannin (10 µmol) given 30 min prior to the addition of 10 µIU insulin and 10 nmol leptin to the samples according to the concentrations described by Aquila *et al.* [1, 2].

2.4 Motility parameters

Motility was measured by means of computer-assisted semen analysis using an Ivos motility analyzer (Hamilton Thorne Biosciences, Beverley, MA, USA) after 1 h, 2 h and 3 h of incubation (37ºC, 5% CO_2).

2.5 Cell viability

Sperm cells that had received different treatments were incubated (37ºC, 5% CO_2, 120 min) and subsequently loaded with propidium iodide (PI) (1 µmol, 15 min). Living cells with an intact cell membrane and active metabolism will exclude PI, whereas cells with damaged membranes or impaired metabolism allow PI to enter the cell and stain the DNA. PI fluorescence was analyzed by fluorescence-activated cell sorting (FACS).

2.6 Acrosome reaction

Spermatozoa that received different treatments were left to capacitate for 3 h, after which they were induced to undergo the acrosome reaction by means of a physiological trigger, progesterone (1 µg/mL, 30 min), or left to undergo the spontaneous acrosome reaction (30 min).

The extent of the acrosome reaction was assessed by placing samples on spotted slides and leaving them to air dry, then fixing them in cold ethanol [20]. Fluorescein isothiocyanate–*Pisum sativum* agglutinin (125 µg/mL) was layered on the slides and they were incubated for 30 min in a dark humid atmosphere. Slides were subsequently rinsed with distilled water in order to eliminate excess probe, then observed under a fluorescence microscope. At least 200 cells were evaluated per spot.

2.7 NO production

NO production was measured as previously described [19]. Briefly, samples that had received different treatments were loaded with DAF-2/DA (10 µmol/L) and incubated (120 min, 37ºC) in the dark. Some of the samples were loaded with the NOS inhibitor, *L*-NAME (0.7 mmol), 30 min prior to DAF-2/DA loading. Care was taken to prevent exposure to light throughout the rest of the experiment as the probe is light-sensitive. After incubation with DAF-2/DA the cells were analyzed by

FACS.

2.8 Flow cytometry

A FACSCalibur analyzer (Becton Dickinson, San Jose, CA, USA) was used to quantify fluorescence (excitation wavelength 488 nm and emission wavelength 530 nm) at a single-cell level and data were analyzed using CellQuest version 3.3 (Becton Dickinson) software. The mean fluorescence intensity of the analyzed sperm cells was determined after gating the cell population by forward and side scatter signals. In total, 100 000 events were acquired, but non-sperm particles and debris were excluded by prior gating, thereby limiting undesired effects on overall fluorescence. The final gated populations usually consisted of 15 000–20 000 sperm cells.

2.9 Statistical analysis

The results were analyzed on the Prism 4 statistical program (GraphPad, San Diego, CA, USA). All data are expressed as mean ± SEM. Data were tested for normality with the Kolmogorov–Smirnov test. One-way ANOVA (with Bonferroni *post hoc* test if $P < 0.05$) was used for statistical analysis. DAF-2/DA fluorescence data are expressed as mean fluorescence (percentage of control, control adjusted to 100%). Differences were regarded statistically significant if $P < 0.05$.

3 Results

3.1 Motility

Total sperm motility, progressive motility, curvilinear velocity (VCL), and amplitude of lateral head displacement (ALH) were assessed after 1, 2 and 3 h of incubation (Figures 1–4, respectively). Leptin as well as insulin + leptin significantly increased total motility compared to the control (75.30 ± 0.57% and 76.10 ± 2.53% *vs.* 64.80 ± 2.74%, respectively; $P < 0.05$) after 1 h of incubation. Similarly, progressive motility was significantly increased in the leptin and insulin + leptin groups compared to the control (51.60 ± 1.98% and 52.30 ± 3.08% *vs.* 42.30 ± 2.84%, respectively; $P < 0.05$). The increase in total motility and progressive motility in the insulin only treated group did not reach significant levels when compared to the control after 1 h of incubation. VCL was significantly increased in the leptin and leptin + insulin groups compared to the control (93.15 ± 2.26 µm/s and 97.40 ± 1.88 µm/s *vs.* 78.51 ± 3.48 µm/s, respectively; $P < 0.05$), whereas ALH was significantly increased in

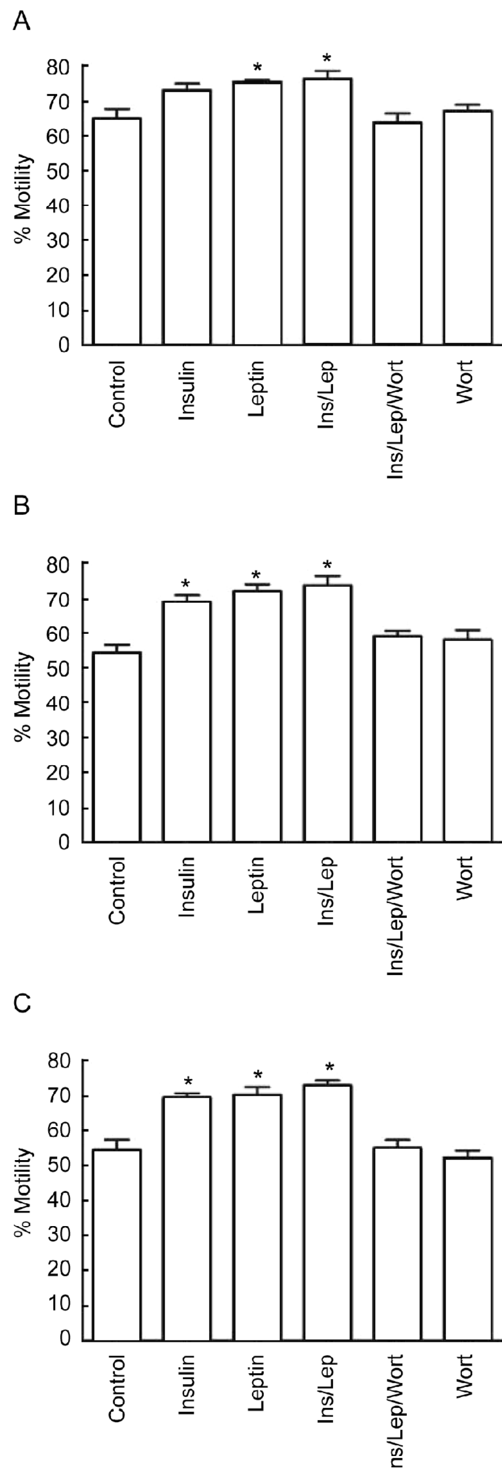

Figure 1. Effects of insulin and leptin on human sperm total motility after 1 (A), 2 (B) and 3 h (C) of incubation. Washed sperm samples were treated with insulin, leptin, insulin + leptin (Ins/Lep), insulin + leptin + wortmannin (Ins/Lep/Wort), or wortmannin (Wort). Values are mean ± SEM of 10 replicates. *$P < 0.05$ *vs.* control.

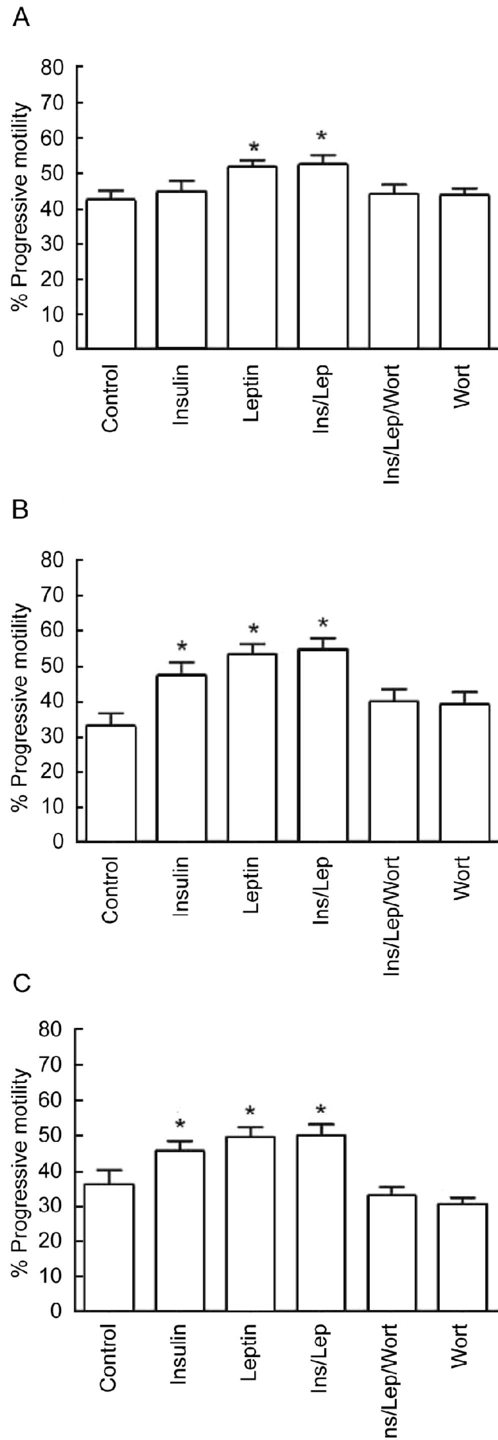

Figure 2. Effects of insulin and leptin on human sperm progressive motility after 1 (A), 2 (B) and 3 h (C) of incubation. Washed sperm samples were treated with insulin, leptin, insulin + leptin (Ins/Lep), insulin + leptin + wortmannin (Ins/Lep/Wort), or wortmannin (Wort). Values are mean ± SEM of 10 replicates. *$P < 0.05$ *vs.* control.

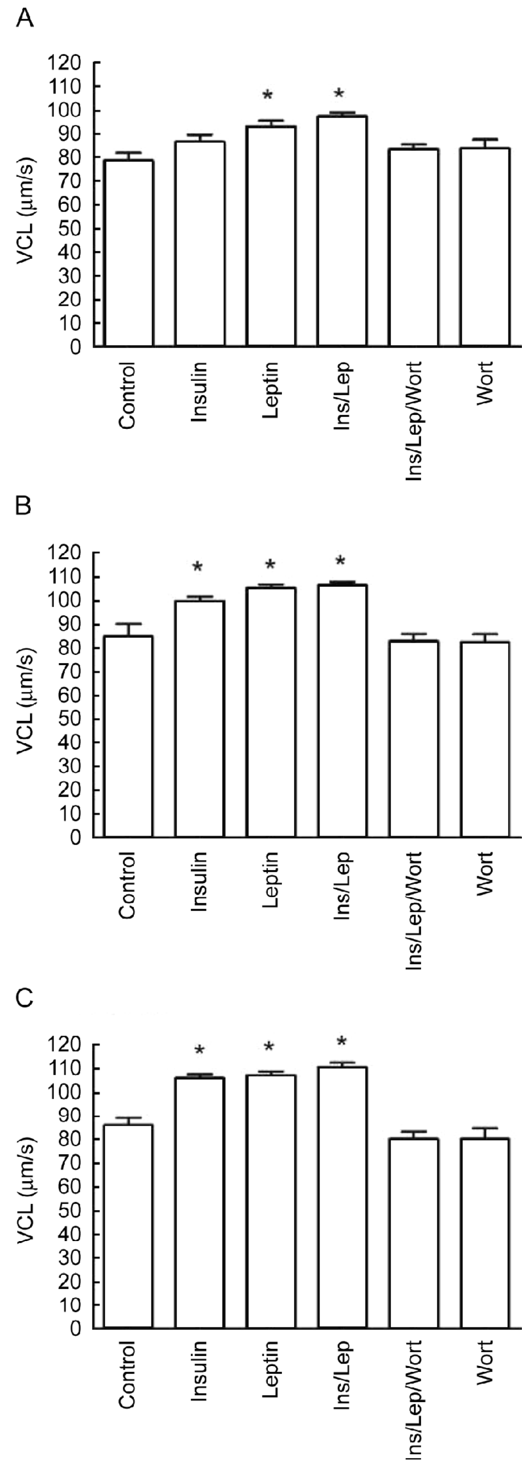

Figure 3. Effects of insulin and leptin on human sperm curvilinear velocity (VCL) after 1 (A), 2 (B) and 3 h (C) of incubation. Washed sperm samples were treated with insulin, leptin, insulin + leptin (Ins/Lep), insulin + leptin + wortmannin (Ins/Lep/Wort), or wortmannin (Wort). Values are mean ± SEM of 10 replicates. *$P < 0.05$ *vs.* control.

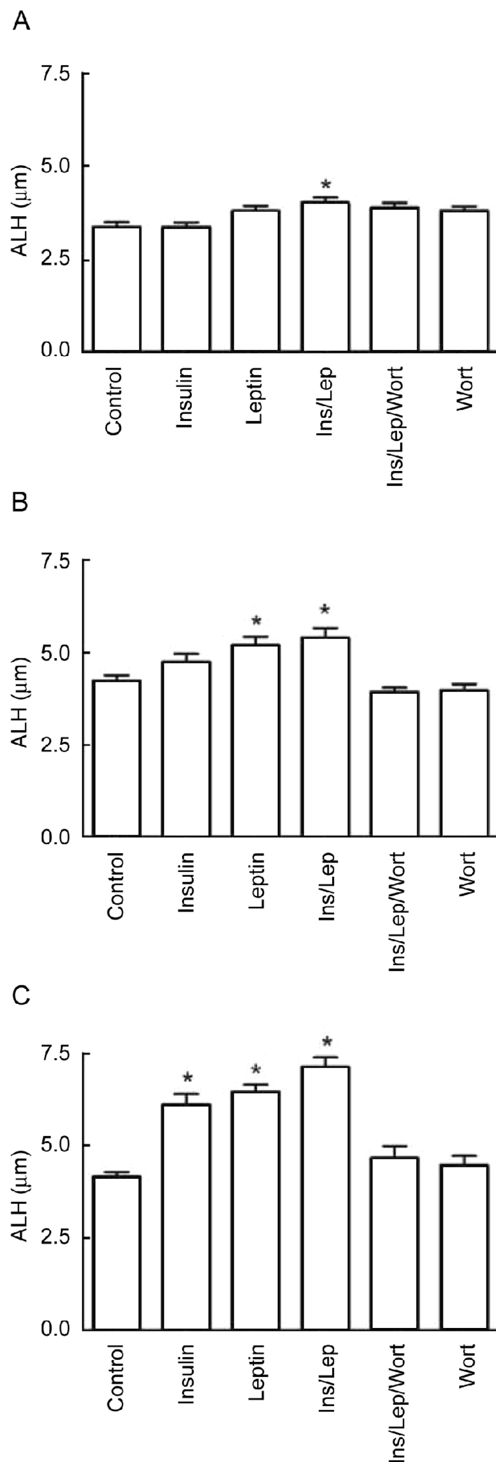

Figure 4. Effects of insulin and leptin on human sperm amplitude of lateral head displacement (ALH) after 1 (A), 2 (B) and 3 h (C) of incubation. Washed sperm samples were treated with insulin, leptin, insulin + leptin (Ins/Lep), insulin + leptin + wortmannin (Ins/Lep/Wort), or wortmannin (Wort). Values are mean ± SEM of 10 replicates. *$P < 0.05$ *vs.* control.

the insulin + leptin group when compared to the control after 1 h of incubation (3.89 ± 0.11 µm *vs.* 3.36 ± 0.13 µm; $P < 0.05$).

After 2 h of incubation, sperm cells incubated with insulin, leptin, or insulin + leptin had significantly increased total motility compared to the control (69.00 ± 2.22%, 72.20 ± 2.02%, and 73.80 ± 2.81% *vs.* 54.30 ± 2.43%, respectively; $P < 0.05$). Similar results were observed with progressive motility. Insulin, leptin, and insulin + leptin groups significantly increased progressive motility compared to the control (47.30 ± 3.81%, 53.20 ± 3.00%, and 54.80 ± 3.13% *vs.* 32.90 ± 3.83%, respectively; $P < 0.05$). The main characteristics of hyperactivation (VCL and ALH) were also significantly increased after 2 h of incubation. VCL was significantly increased in the insulin, leptin, and insulin + leptin groups compared to the control (99.78 ± 2.07 µm/s, 105.2 ± 1.87 µm/s, and 106.6 ± 1.59 µm/s *vs.* 84.97 ± 5.39 µm/s, respectively; $P < 0.05$). However, ALH was significantly increased in the leptin and insulin + leptin groups when compared to the control (5.20 ± 0.24 µm and 5.40 ± 0.26 µm *vs.* 4.23 ± 0.13 µm, respectively; $P < 0.05$).

A similar trend of events was observed after 3 h of incubation. The insulin, leptin, and insulin + leptin groups had significantly increased total motility, progressive motility, and VCL, as well as ALH, when compared to the control.

At all time points the addition of wortmannin did not affect motility, however, it was able to attenuate the effects of insulin/leptin on motility, progressive motility, VCL, and ALH when used as a cotreatment.

3.2 *Sperm cell viability*

We observed a trend of decreased PI fluorescence, interpreted as an increase in viability, for cells treated with insulin, leptin, and insulin + leptin, but it did not attain statistical significance (Figure 5).

3.3 *Acrosome reaction*

Progesterone-stimulated samples had significantly more acrosome-reacted cells compared to spontaneous acrosome-reacted cells in all the groups (Figure 6). The addition of insulin, leptin, and insulin + leptin significantly increased spontaneous acrosome-reacted cells compared to the control (35.33 ± 1.73%, 36.56 ± 1.93%, and 41.78 ± 1.31% *vs.* 14.56 ± 0.64%, respectively; $P < 0.05$). Similarly, insulin, leptin, and insulin + leptin significantly increased acrosome reaction in cells stimulated with proges-

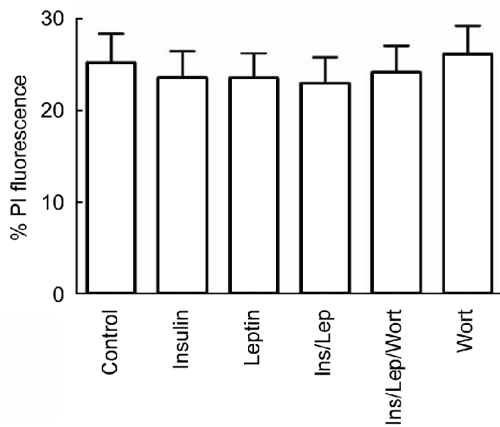

Figure 5. Effects of insulin and leptin on propidium iodide (PI) fluorescence. PI was used as a probe for non-viable cells. Spermatozoa were treated with insulin, leptin, insulin + leptin (Ins/Lep), insulin + leptin + wortmannin (Ins/Lep/Wort), or wortmannin (Wort). Data is expressed as the percentage of PI fluorescence.

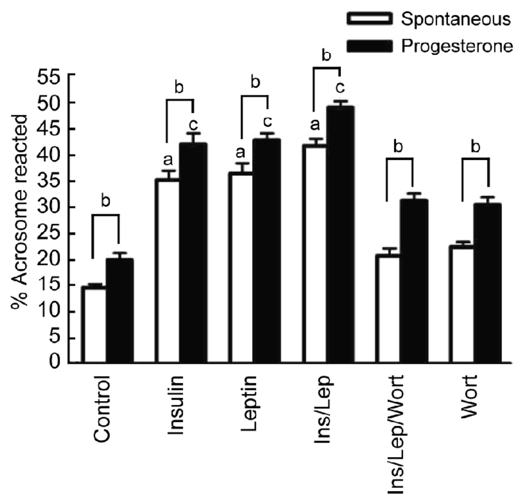

Figure 6. Effects of insulin and leptin on sperm acrosome reaction. Spermatozoa were treated with insulin, leptin, insulin + leptin (Ins/Lep), insulin + leptin + wortmannin (Ins/Lep/Wort), or wortmannin (Wort). Cells were simulated to acrosome react with progesterone or left to undergo spontaneous acrosome reaction. [a]$P < 0.05$ *vs*. spontaneous control; [b]$P < 0.05$ *vs*. spontaneous; [c]$P < 0.05$ *vs*. progesterone control.

terone when compared to the control (42.11 ± 2.05%, 42.89 ± 1.26%, and 49.11 ± 1.18% *vs*. 20.00 ± 1.35%, respectively; $P < 0.05$). The inhibition of PI3K with wortmannin did not affect the percentage of acrosome-reacted cells compared to the control in either spontaneous or progesterone-stimulated groups. Wortmannin, however, attenuated the stimulatory effects of insulin/leptin on acrosome reaction when used as a cotreatment.

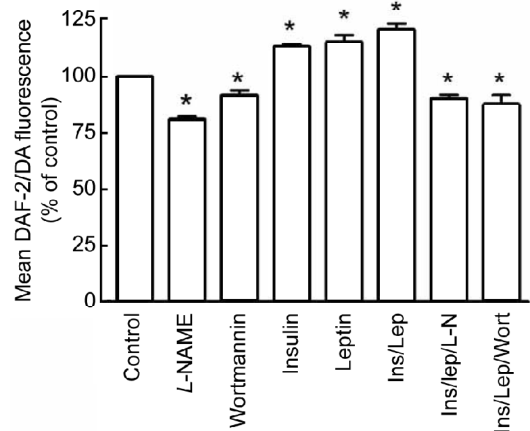

Figure 7. Effects of insulin and leptin on 4,5-diaminofluorescein-2/diacetate (DAF-2/DA) fluorescence. Spermatozoa were treated with *N*-nitro-*L*-arginine methyl ester (*L*-NAME), wortmannin, insulin, leptin, insulin + leptin (Ins/Lep), insulin + leptin + *L*-NAME (Ins/Lep/L-N), insulin + leptin + wortmannin (Ins/Lep/Wort). Values are expressed as mean DAF-2/DA fluorescence percentage of the control (control adjusted to 100%) of 10 replicates. *$P < 0.05$ *vs*. control.

3.4 NO generation

Figure 7 shows the effects of insulin and leptin on DAF-2/DA fluorescence. The NOS inhibitor, *L*-NAME, significantly reduced DAF-2/DA fluorescence compared to the control (81.01 ± 1.48% *vs*. 100%; $P < 0.05$). Wortmannin, a PI3K inhibitor, also significantly reduced DAF-2/DA fluorescence compared to the control (91.58 ± 2.35% *vs*. 100%; $P < 0.05$). Insulin, leptin, and insulin + leptin groups significantly increased DAF-2/DA fluorescence compared to the control (113.10 ± 1.25%, 115.30 ± 3.24%, and 120.80 ± 2.70% *vs*. 100%, respectively; $P < 0.05$). The addition of insulin + leptin to the *L*-NAME and wortmannin treated groups did not reverse the situation.

4 Discussion

The existence of insulin and leptin in human ejaculated spermatozoa was shown through their transcripts evaluated by reverse transcription–polymerase chain reaction, their protein content evidenced by Western blot

analysis and through their localization by immunostaining analysis [1, 2]. The significance of leptin in influencing reproduction was evidenced by leptin-deficient female mice (*ob* mice) that are infertile [21]. However, treatment with leptin restores fertility in *ob* male mice, suggesting its role in reproduction [22]. The role of leptin in human spermatozoa function is not clearly elucidated. Most studies have indicated both positive and negative effects of leptin in gonadal function [23, 24]. Glander *et al.* [25] reported that seminal plasma leptin levels were significantly lower in patients with normal spermiogram parameters, compared with pathological semen samples, and showed a negative correlation with motility of human spermatozoa, suggesting that higher leptin concentration has negative effects on sperm function. However, Zorn *et al.* [26] found no correlation between leptin levels and sperm motility or morphology.

The importance of insulin in spermatozoa physiology is indicated by men affected by diabetes mellitus type 1 who have sperm with severe structural defects, significantly lower motility [27] and lower ability to penetrate hamster eggs [28]. Our data has shown that insulin and leptin might play a role in enhancing human sperm motility parameters, as evidenced by increased total and progressive motility as well as the sperm hyperactivation characteristics, VCL and ALH (Figures 1–4).

Insulin and leptin secretion was reported to be significantly increased in capacitated sperm than in non-capacitated sperm, suggesting the involvement of these hormones in capacitation. Capacitated sperm released up to approximately 18 μIU insulin and 4 ng/mL leptin [1, 2]. Lackey *et al.* [29] reported leptin concentration levels of approximately 1 ng/mL in human seminal plasma, whereas in female follicular fluid, leptin levels of approximately 16 ng/mL have been reported [30].

Studies have shown that capacitated sperm display an increase in metabolic rate, overall energy expenditure, intracellular ion concentrations, plasma membrane fluidity, intracellular pH, and reactive oxygen species, presumably to affect the changes in sperm signaling and function during capacitation [31, 32]. Sperm capacitation is a prerequisite step for sperm to undergo the acrosome reaction [33, 34]. This possibly explains why insulin and leptin increased the percentage of spontaneous and progesterone acrosome-reacted cells in our study. It is not clear whether this increase is due to the agonists' effect on capacitation or acrosome reaction itself. Further studies are recommended. However, the blockage of PI3K with wortmannin had no effect on the acrosome reaction status of the cells when compared to the control. This finding is consistent with results observed by Fisher *et al.* [35], in which wortmannin was found not to inhibit the acrosome reaction induced by A23187 or progesterone, as well as by du Plessis *et al.* [36], where LY294002, another PI3K inhibitor, also did not inhibit the acrosome reaction induced by A23187, progesterone, and solubilized zona pellucida. We speculate that the cellular pathways involved in the acrosome reaction induced by this agonist do not involve PI3K, or, alternatively, that the need for PI3K in the pathway is somehow by-passed. It has been reported that the signaling of insulin is a complex process that involves multiple signaling pathways that diverge at or near the activation of its tyrosine kinase receptor [37].

Studies have reported that insulin and leptin enhance NO production in other cell types [38, 39]. Our study has, for the first time, shown that both insulin and leptin enhance NO production in human spermatozoa and that this increase is possibly through the PI3K signaling pathway, as evidenced by reduction of NO production when the PI3K inhibitor, wortmannin, was given. However, it is still too early to make significant conclusions about the mechanism of action of insulin and leptin on NO production, as wortmannin has also been shown to inhibit phosphotidylinositol 4-kinase [40]. The attenuation of NO production when the NOS inhibitor, *L*-NAME, was given confirms that the NO was derived from NOS (Figure 7).

In conclusion, our study has shown that insulin and leptin might play a role in enhancing the fertilization capacity of human spermatozoa by increasing motility, acrosome reaction, and NO production.

Acknowledgment

We would like to thank the Harry Crossly Foundation, University of Stellenbosch, and Malawi College of Medicine NORAD project for funding.

References

1 Aquila S, Gentile M, Middea E, Calatano S, Ando S. Autocrine regulation of insulin secretion in human ejaculated spermatozoa. Endocrinology 2005; 146: 552–7.

2 Aquila S, Gentile M, Middea E, Catalano S, Morelli C, Pezzi V, *et al*. Leptin secretion by human ejaculated spermatozoa. J Clin Endocrinol Metab 2005; 90: 4753–61.

3 Campfield LA, Smith FJ, Burn P. The OB protein (leptin) pathway. A link between adipose tissue mass and central neural networks. Horm Metab Res 1996; 28: 619–31.

4 Schwartz MW, Baskin DG, Kaiyala KJ, Woods SC. Model for the regulation of energy balance and adiposity by the central nervous system. Am J Clin Nutr 1999; 69: 584–96.

5 Dallongeville J, Fruchart JC, Auwerx J. Leptin, a pleiotropic hormone: physiology, pharmacology and strategies for discovery of leptin modulators. J Med Chem 1998; 41: 5337–52.

6 Cunningham MJ, Clifton DK, Steiner RA. Leptin's action on the reproductive axis: perspectives and mechanisms. Biol Reprod 1999; 60: 216–22.

7 Quinton ND, Smith RF, Clayton PE, Gill MS, Shalet S, Justice SK, *et al*. Leptin binding activity changes with age: the link between leptin and puberty. J Clin Endocrinol Metab 1999; 84: 2336–41.

8 Wauters M, Considine RV, van Gaal LF. Human leptin: from adipocyte hormone to an endocrine mediator. Eur J Endocrinol 2000; 143: 293–311.

9 Jope T, Lammert A, Kratzsch J, Paasch U. Leptin and leptin receptor in human seminal plasma and human spermatozoa. Int J Androl 2003; 26: 335–41.

10 Philippe J. Structure and pancreatic expression of the insulin and glucagon genes. Endocr Rev 1991; 12: 252–71.

11 Ali ST, Shaikh RN, Siddiqi NA, Siddiqi PQ. Semen analysis in insulin-dependent/non-insulin-dependent diabetic men with/without neuropathy. Arch Androl 1993; 30: 47–54.

12 Aiston S, Agius L. Leptin enhances glycogen storage in hepatocytes by inhibition of phosphorylase and exerts an additive effect with insulin. Diabetes 1999; 48: 15–20.

13 Middendorff R, Muller D, Wichers S, Holstein AF, Davidoff MS. Evidence for production and functional activity of nitric oxide in seminiferous tubules and blood vessels of the human testis. J Clin Endocrinol Metab 1997; 82: 4154–61.

14 Thundathil J, de Lamirande E, Gagnon C. Nitric oxide regulates the phosphorylation of the threonine–glutamine–tyrosine motif in proteins of human spermatozoa during capacitation. Biol Reprod 2003; 68: 1291–8.

15 O'Bryan MK, Zini A, Cheng CY, Schlegel PN. Human sperm endothelial nitric oxide synthase expression: correlation with sperm motility. Fertil Steril 1998; 70: 1143–7.

16 Lewis SE, Donnelly ET, Sterling ES, Kennedy MS, Thompson W, Chakravarthy U. Nitric oxide synthase and nitrite production in human spermatozoa: evidence that endogenous nitric oxide is beneficial to sperm motility. Mol Hum Reprod 1996; 2: 873–8.

17 Revelli A, Soldati G, Costamagna C, Pellarey O, Aldieri E, Massobrio M, *et al*. Follicular fluid proteins stimulate nitric oxide (NO) synthesis in human sperm: a possible role for NO in acrosomal reaction. J Cell Physiol 1999; 178: 85–92.

18 Donnelley T, Lewis SE, Thompson W, Chakravarthy U. Sperm nitric oxide and motility: the effects of nitric oxide synthase stimulation and inhibition. Mol Hum Reprod 1997; 3: 755–62.

19 Lampiao F, Strijdom H, du Plessis SS. Direct nitric oxide measurement in human spermatozoa: flow cytometric analysis using the fluorescent probe, diaminofluorescein. Int J Androl 2006; 29: 564–7.

20 World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction, 4th edn. Cambridge: Cambridge University Press; 1999.

21 Jones N, Harrison GA. Genetically determined obesity and sterility in the mouse. Proc Soc Study Fertil 1957; 9: 51–64.

22 Chehab FF, Lim ME, Lu R. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nat Genet 1996; 12: 318–20.

23 Caprio M, Fabbrini E, Isidori AM, Aversa A, Fabbri A. Leptin in reproduction. Trends Endocrinol Metab 2001; 12: 65–72.

24 Clarke IJ, Henry BA. Leptin and reproduction. Rev Reprod 1999; 4: 48–55.

25 Glander HJ, Lammert A, Paasch U, Glasow A, Kratzsch J. Leptin exists in tubuli seminiferi and in seminal plasma. Andrologia 2002; 34: 227–33.

26 Zorn B, Osredkar J, Meden-Vrtovec H, Majdic G. Leptin levels in infertile male patients are correlated with inhibin B, testosterone and SHBG but not with sperm characteristics. Int J Androl 2007; 30: 439–44.

27 Baccetti B, La Marca A, Piomboni P, Capitani S, Bruni E, Petraglia F, *et al*. Insulin-dependent diabetes in men is associated with hypothalamo-pituitary derangement and with impairment in semen quality. Hum Reprod 2002; 17: 2673–7.

28 Shrivastav P, Swann J, Jeremy JY, Thompson C, Shaw RW, Dandona P. Sperm function and structure and seminal plasma prostanoid concentrations in men with IDDM. Diabetes Care 1989; 12: 742–4.

29 Lackey BR, Gray SL, Henricks DM. Measurement of leptin and insulin-like growth factor-1 in seminal plasma from different species. Physiol Res 2002; 51: 309–11.

30 Dorn C, Reinsberg J, Kupka M, van der Ven H, Schild RL. Leptin, VEGF, IGF-1, and IGFBP-3 concentrations in serum and follicular fluid of women undergoing *in vitro* fertilization. Arch Gynecol Obstet 2003; 268: 187–93.

31 Aitken RJ, Nixon B, Lin M, Koppers AJ, Lee YH, Baker MA. Proteomic changes in mammalian spermatozoa during epididymal maturation. Asian J Androl 2007; 9: 554–64.

32 Visconti PE, Galantino-Homer H, Moore GD, Bailey JL, Ning X, Fornes M, *et al*. The molecular basis of sperm capacitation. J Androl 1998; 19: 242–8.

33 Liu RZ, Na WL, Zhang HG, Lin ZY, Xue BG, Xu ZG. Assessment of released acrosin activity as a measurement of the sperm acrosome reaction. Asian J Androl 2008; 10: 236–342.

34 Jaiswal BS, Cohen-Dayag A, Tur-Kaspa I, Eisenbach M. Sperm capacitation is, after all, a prerequisite for both partial and complete acrosome reaction. FEBS Lett 1998; 427: 309–13.

35 Fisher HM, Brewis IA, Barratt CL, Cooke ID, Moore HD. Phosphoinositide 3-kinase is involved in the induction of the human sperm acrosome reaction downstream of tyrosine phosphorylation. Hum Reprod 1998; 4: 849–55.

36 du Plessis SS, Franken DR, Baldi E, Luconi M. Phosphatidylinositol

3-kinase inhibition enhances human sperm motility and sperm–zona pellucida binding. Int J Androl 2004; 27: 19–26.
37 Saltiel AR, Pessin J. Insulin signaling in time and space. Trends Cell Biol 2002; 12: 65–71.
38 White V, Gonzalez E, Capobianco E, Pustovrh C, Martinez N, Higa R, *et al*. Leptin modulates nitric oxide production and lipid metabolism in human placenta. Reprod Fertil Dev 2006; 18: 425–32.
39 Kim JW. Insulin enhances nitric oxide production in trabecular meshwork cells via *de novo* pathway for tetrahydrobiopterin synthesis. Korean J Ophthalmol 2007; 21: 39–44.
40 Etkovitz N, Rubinstein S, Daniel L, Breitbart H. The role of PI3-kinase and PI4-kinase in actin polymerization during bovine sperm capacitation. Biol Reprod 2007; 77: 263–73.

Edited by Prof Ralf Henkel

Register 3APFA now!

We are very pleased to inform you that **Third Asia-Pacific Forum on Andrology (3APFA), in conjunction with the Tenth Anniversary Celebration of Asian Journal of Andrology**, will be held in Nanjing, China on October 10–13, 2009. The theme of this conference is Environment, Life Style & Genetic/Epigenetic Factors and Men's Health

Please complete registration through the Forum website at **http://www.asiaandro.com/3APFA**. If online registration is not available, please consult the 3APFA secretariat by email or phone. The hard copy of registration is only acceptable for those who could not access to the Internet or Group Registration. Applications for registration not accompanied by the appropriate fee will not be accepted. The on-line registration system will close at 18:00 on Sep 30, 2009 (local time). All registrations received after this time will be considered as on-site registration. For the benefit of lower registration rates, it is strongly recommended to make early registration by May 31, 2009, and late registration from June 1 to Sep 30, 2009.

Registration fee:

Category	Early Registration <By May 31, 2009>	Late Registration <June 1–Sep 30, 2009>	On-Site Registration
Regular Participant	USD650	USD700	USD750
Student[1]	USD250	USD300	USD350
Accompanying Person[2]	USD300	USD300	USD300
Group[3](10 or more)	USD520	USD560	USD600

[1]The Student Certificate must be provided at the on-site registration table, otherwise the full registration will be charged.
[2]Accompanying persons are limited to family members and not allowed to attend scientific sessions.
[3]Students and accompanying persons can not be included in Group registration. Please visit Forum Website or contact the Secretatirat for further informtion on **Group Registration**.

Online Registration: http://www.asiaandro.com/3APFA/login.asp
Online Registration dealine: Sep 30, 2009

3APFA 2009 Secretariat
Ms. Dan-Qing Ren or Ms. Hui Zhang
Editorial Office, Asian Journal of Andrology, 294 Tai-yuan Rd, Shanghai 200031, China
Phone: +86-21-5492 2824
Fax: +86-21-5492 2825
E-mail: apfa@sibs.ac.cn; aja@sibs.ac.cn; aja@mail.shcnc.ac.cn